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# FURTHER STUDIES ON THE TREATMENT OF CONGENITAL ADRENAL HYPERPLASIA WITH CORTISONE

## I. COMPARISON OF ORAL AND INTRAMUSCULAR ADMINISTRATION OF CORTISONE, WITH A NOTE ON THE SUPPRESSIVE ACTION OF COMPOUNDS F AND B ON THE ADRENAL*†

LAWSON WILKINS, M.D., LYTT I. GARDNER, M.D., JOHN F. CRIGLER, JR., M.D., SAMUEL H. SILVERMAN, M.D. AND CLAUDE J. MIGEON, M.D.‡

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PREVIOUSLY published studies (1, 2, 3) have shown that the administration of cortisone to patients with congenital adrenal hyperplasia caused markedly decreased excretion of 17-ketosteroids and biologically active androgen. Both chemical tests and bioassays showed that there was a simultaneous decrease in urinary estrogen, presumably derived from the adrenals. The output of "corticosteroids" was less uniformly affected, probably due to the fact that while the endogenous secretion of these steroids was suppressed a portion of the cortisone was excreted

Received for publication November 26, 1951.

* Parts of these studies were reported in abstract at the Annual Meeting of the Association of American Physicians in Atlantic City, N. J., May, 1951; and at the Annual Meeting of the Association for the Study of Internal Secretions, Atlantic City, June 7, 1951.

† This work was made possible by a Grant-in-Aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council, and by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

‡ Fellow of the André and Bella Meyer Foundation (France) on the recommendation of the Institute of International Education, New York.

TABLE 1. PATIENTS WITH CONGENITAL ADRENAL HYPERPLASIA, UNDER CONTINUOUS TREATMENT WITH CORTISONE FOR 5–17 MONTHS

| Series No.* | Name and HLH No. | Age at beginning of Rx | Wt., Kg. | Associated symptoms | 17-KS** before Rx, mg./day | Treatment with Cortisone | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Date begun | Route | Average daily dose, mg. | How given | 17-KS,** mg./day | Total duration (months) |
| *Group A—Older Female Pseudohermaphrodites* | | | | | | | | | | | |
| 5 | M.B. A-47344 | 8½ yrs. | 40 | | 38 | 6- 5-50 | i.m. | 100.0 | 100.0 q d | 5.7 | 17 |
| | | | | | | 6-11-50 | i.m. | 25.0 | 25.0 q d | 5.3 | |
| | | | | | | 10- 8-50 | i.m. | 25.0 | 50.0 q 2 d | 5.7 | |
| | | | | | | 2-27-51 | i.m. | 16.6 | 50.0 q 3 d | 9.6 | |
| | | | | | | 5-16-51 | i.m. | 25.0 | 75.0 q 3 d | 6.2 | |
| | | | | | | 10- 7-51 | i.m. | 25.0 | 100.0 q 4 d | 7.4 | |
| 7 | J.S. 92456 | 18½ yrs. | 50 | Hypertension; pigmentation | 53 | 6-19-50 | i.m. | 50.0 | 50.0 q d | 4.5 | 17 |
| | | | | | | 7-17-50 | i.m. | 25.0 | 50.0 q 2 d | 5.0 | |
| | | | | | | 11- 4-50 | i.m. | 25.0 | 75.0 q 3 d | 4.8 | |
| | | | | | | 1- 6-51 | i.m. | 16.6 | 50.0 q 3 d | 9.6 | |
| | | | | | | 6-11-51 | i.m. | 25.0 | 75.0 q 3 d | 3.8 | |
| | | | | | | 8-28-51 | i.m. | 25.0 | 100.0 q 4 d | 5.6 | |
| 9 | M.C.S. 92457 | 16½ yrs. | 51 | Hypertension; pigmentation | 62 | 11- 4-50 | o | 50.0 | 25.0×2 | 21.0 | 12 |
| | | | | | | 11-14-50 | o | 50.0 | 12.5×4 | 17.0 | |
| | | | | | | 11-19-50 | i.m. | 50.0 | 50.0 q d | 8.0 | |
| | | | | | | 11-30-50 | o | 50.0 | 25.0×2 | 6.0 | |
| | | | | | | 1- 9-51 | o | 25.0 | 12.5×2 | 13.4 | |
| | | | | | | 3- 4-51 | i.m. | 25.0 | 25.0 q d | 4.8 | |
| | | | | | | 6-11-51 | i.m. | 25.0 | 75.0 q 3 d | 5.0 | |
| 1 | P.R. A-58433 | 16 yrs. | 66 | | 50 | 11-12-50 | o | 100.0 | 50.0×2 | 7.0 | 12 |
| | | | | | | 11-28-50 | o | 25.0 | 12.5×2 | 19.0 | |
| | | | | | | 12- 5-50 | o | 50.0 | 25.0×2 | 13.0 | |
| | | | | | | 4- 6-51 | o | 75.0 | 25.0×3 | 5.0 | |
| 3 | W.D. A-30453 | 9¼ yrs. | 41 | | 30 | 2- 3-51 | o | 25.0 | 12.5×2 | 22.0 | 9 |
| | | | | | | 2-18-51 | o | 37.5 | 12.5×3 | 13.0 | |
| | | | | | | 2-28-51 | o | 50.0 | 25.0×2 | 4.0 | |
| 4 | M.N. A-76987 | 10½ yrs. | 39 | | 34 | 5-25-51 | i.m. | 25.0 comp. F | 25.0 q d | 12.0 | 5 |
| | | | | | | 6-15-51 | o | 50.0 cortisone | 25.0×2 | 5.0 | |
| *Group B—Young Infants with Female Pseudohermaphrodism and Salt Loss* | | | | | | | | | | | |
| 8 | L.U. A-79277 | 11 wks. | 3.6 | Salt loss | 3.5 | 8-14-50 | i.m. | 25.0 | 25.0 q d | 0.4 | 15 |
| | | | | | | 12- 5-50 | i.m. | 5.0 | 5.0 q d | 0.3 | |
| | | | | | | 2-14-51 | o | 5.0 | 2.5×2 | 0.6 | |
| | | | | | | 3-29-51 | — | 0.0 | — | 3.3 | |
| | | | | | | 4-30-51 | o | 5.0 | 2.5×2 | 1.2 | |
| | | | | | | 5-21-51 | — | 0.0 | — | 2.9 | |
| | | | | | | 6-13-51 | i.m. | 12.5 comp. B | 12.5 q d | 1.3 | |
| | | | | | | 8-24-51 | i.m. | 25.0 comp. B | 25.0 q d | 1.0 | |
| | | | | | | 9- 5-51 | i.m. | 5.0 comp. B | 5.0 q d | 1.8 | |
| | | | | | | 9-21-51 | — | 0.0 | — | 6.0 | |
| | | | | | | 9-28-51 | o | 12.0 | 4.0×3 | 1.7 | |
| 10 | F.F. A-81728 | 19 wks. | 5.3 | Salt loss | 4.3 | 1- 3-51 | o | 25.0 | 12.5×2 | 1.4 | 10 |
| | | | | | | 1-29-51 | o | 12.0 | 6.0×2 | 1.1 | |
| 11 | S.K. A-83131 | 11 wks. | 4.2 | Salt loss | 3.2 | 2- 9-51 | o | 12.0 | 6.0×2 | 1.7 | 9 |
| | | | | | | 2-28-51 | i.m. | 12.5 | 12.5 q d | 0.5 | |
| | | | | | | 3-23-51 | i.m. | 8.3 | 25.0 q 3 d | 0.3 | |
| | | | | | | 6-12-51 | i.m. | 6.25 | 25.0 q 4 d | 1.3 | |
| *Group C—Males with Macrogenitosomia Praecox* | | | | | | | | | | | |
| 2 | W.J. A-68429 | 4½ yrs. | 25 | Hypertension; cardiac failure | 8.6 | 12-22-50 | i.m. | 12.5 | 12.5 q d | 1.3 | 10 |
| | | | | | | 6-16-51 | o | 25.0 | 12.5×2 | 1.2 | |
| 12 | T.T. A-87946 | 6 10/12 yrs. | 27 | | 26 | 5- 8-51 | i.m. | 100.0 | 100.0 q d | 3.8 | 6 |
| | | | | | | 5-16-51 | i.m. | 25.0 | 50.0 q 2 d | 2.9 | |
| | | | | | | 6-11-51 | i.m. | 25.0 | 75.0 q 3 d | 3.5 | |

* Series numbers coincide with those of patients reported previously (J. Clin. Endocrinol. 11: 1, 1951).
** Values of 17-ketosteroids represent averages of all determinations during each period.

in this fraction. The patients were all treated with cortisone injected intramuscularly. The last report dealt with 8 patients, 6 females with pseudohermaphrodism and 2 males with macrogenitosomia praecox. At the time of its publication 2 patients had been treated continuously for five months, 1 for three months, the others for periods of only ten to fifty-eight days. It was found that initial doses of 50 mg. or 25 mg. of intramuscular cortisone per day were as effective as 100 mg. per day in causing adrenal suppression. It appeared that 25 mg. of intramuscular cortisone daily would maintain this suppression in the older patients and that probably considerably smaller amounts would be required in infants. In this series, the 2 female pseudohermaphrodites (aged $8\frac{1}{2}$ and $18\frac{1}{2}$ years) who had been treated for five months had developed breasts, and the older had had regular menstrual cycles. Decreased growth of sexual hair and lessened acne suggested that virilization was diminished. With the doses of cortisone administered, no changes in carbohydrate metabolism were observed. Most of the patients showed no symptoms suggestive of excessive cortisone effect. One boy of $2\frac{1}{2}$ years, however, given 50 mg. of cortisone daily for ten days, became euphoric and manic and his previously existing hypertension increased. Another infant of 11 weeks, after receiving 25 mg. of cortisone daily for two or three months, became obese with a round "moon face" suggestive of Cushing's syndrome.

On the basis of the above observations it was considered advisable to begin continuous treatment with cortisone of those patients who had received it previously only for short periods of study. A number of other patients with congenital adrenal hyperplasia were also available for treatment, but the relatively limited facilities of the laboratory and metabolism ward have permitted us to begin treatment of only 3 new patients. The simplicity of treatment with cortisone given orally made it seem desirable to compare the minimum doses of oral and intramuscular cortisone which would maintain indefinitely adequate suppression of the overactive adrenals without causing undesirable adverse metabolic or toxic effects. Furthermore, the effect of medication on the 17-ketosteroid excretion offered an entirely objective method of comparing quantitatively the efficiency of the oral and intramuscular administration of cortisone, in regard to its ability to suppress hyperactivity of the adrenals. Such an objective comparison is not available in the treatment of rheumatoid arthritis and other disorders.

The present report is based on observations made on 11 patients treated continuously for periods ranging from five to seventeen months. On the basis of their age and sex they fall into 3 groups (Table 1). Group A consists of 6 female pseudohermaphrodites over 8 years of age. Group B consists of 3 female pseudohermaphrodites with defects of electrolyte regula-

tion who were between 11 and 19 weeks of age when first treated with cortisone. Group C consists of 2 boys with macrogenitosomia praecox, aged 4½ and 7 years. The findings will be discussed from the standpoint of comparing the effective suppressive dose of cortisone given orally and intramuscularly. In addition we shall discuss the effects of compound F and of compound B in 1 patient each. The clinical effects of treatment and the influence of cortisone on the electrolyte metabolism and on hypertension will be dealt with in separate papers.

The comparison between the oral and intramuscular administration of cortisone was facilitated by the fact that 3 of the patients (Cases 1, 3 and 4) who were given cortisone *orally* in the present study had been treated eleven or twelve months previously with *intramuscular* cortisone for periods of only fifteen to fifty-eight days. In addition, 2 sisters (Cases 7 and 9) afforded an excellent opportunity to compare intramuscular and oral therapy. The data are summarized in Table 1.

## DOSAGE OF INTRAMUSCULAR AND ORAL CORTISONE IN OLDER PATIENTS (GROUP A)

### *Level of urinary 17-ketosteroids indicating maximal adrenal suppression*

It seemed desirable to attempt to determine the minimum dose of cortisone which would suppress as much as possible the secretion of adrenal androgen. In previous studies with intramuscular cortisone it was found that in patients of the older age group (Nos. 1, 3, 4, 5 and 7) the daily excretion of urinary 17-ketosteroids could be reduced to levels which fluctuated between 3 and 8 mg. The averages in different cases, calculated over long periods, were from 4.3 to 7.1 mg. per day. Even when the dose of intramuscular cortisone was raised to 100 mg. per day, the urinary 17-ketosteroids did not fall to levels lower than these. For this reason we believe that sufficient cortisone should be administered to suppress the 17-ketosteroids to 8 mg. per day or less. Likewise it is our clinical impression that when the output of 17-ketosteroids exceeds this amount, reaching perhaps between 9 and 12 mg. per day, the suppression of virilization indicated by decreased hair growth and disappearance of acne was less complete. Therefore, in the older age group now under discussion we have attempted to compare the minimum doses of cortisone administered intramuscularly and orally which are capable of maintaining the urinary 17-ketosteroids *below 8 mg. per day*. This level is indicated on the charts by dotted lines. In long continued therapy it is obvious that it is desirable to give the smallest effective dose of cortisone in order to avoid as much as possible the risk of abnormal metabolic or toxic effects.

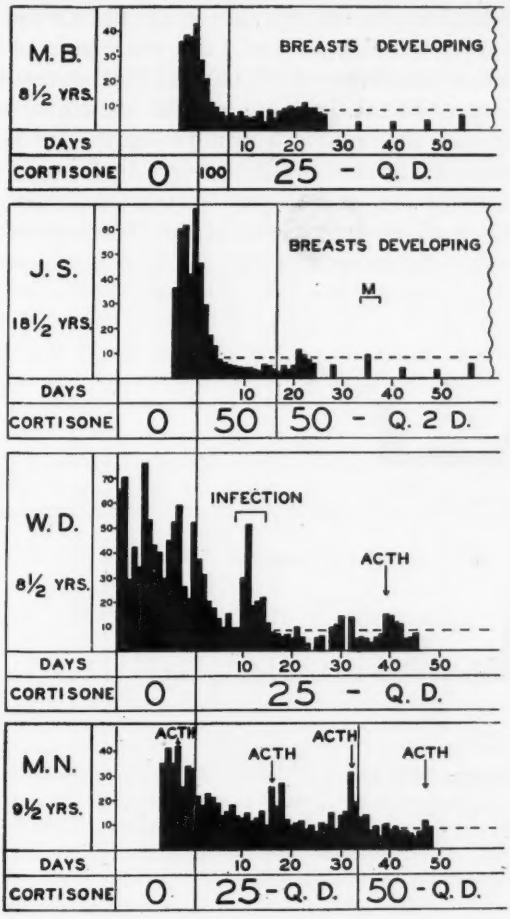


FIG. 1. Comparison of the effects of initial doses of 100 mg., 50 mg. and 25 mg. of cortisone on the suppression of urinary 17-ketosteroids of 4 female pseudohermaphrodites.

*Initial dose required to bring about full suppression*

Figure 1 shows a comparison of initial daily doses of 100 mg., 50 mg. and 25 mg. of cortisone given intramuscularly. In the cases of M. B., J. S. and W. D., initial doses of 100 mg., 50 mg. and 25 mg. of cortisone injected intramuscularly once a day were equally effective in suppressing the excretion of 17-ketosteroids. However, in patient M. N., 25 mg.

reduced the 17-ketosteroids only to an average of 11.8 per day, whereas 50 mg. per day lowered the output to 7.1 mg. per day.

Figure 2 shows a comparison of the effects of intramuscular and of oral cortisone in the case of P. R. In January 1950, 100 mg. of cortisone given intramuscularly each day caused a prompt decrease in the urinary 17-ketosteroids below the level of 8 mg. per day. In January 1951, the oral administration of 100 mg. of cortisone daily given in two divided doses had an equal effect on the suppression of the 17-ketosteroids.

Figure 3 shows a comparison of the effects of intramuscular and oral

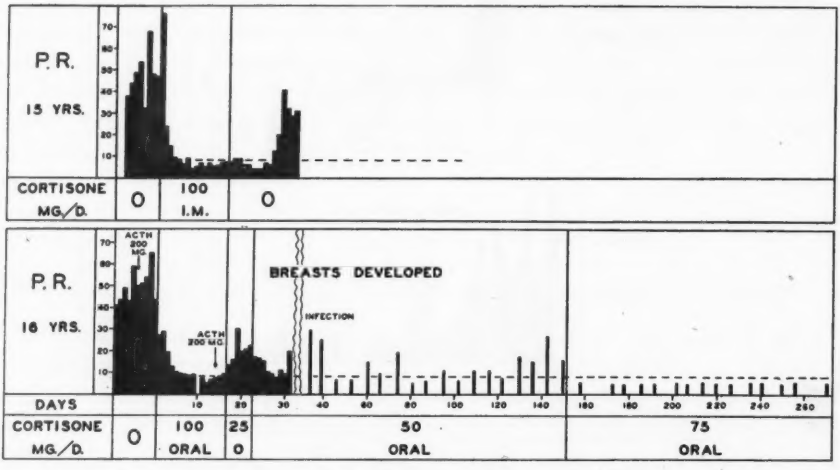


FIG. 2. Comparison of the effects of intramuscular and oral cortisone on the supression of urinary 17-ketosteroids of a female pseudohermaphrodite.

In doses of 100 mg. daily both oral and intramuscular cortisone caused prompt suppression, but 75 mg. of oral cortisone daily (25 mg. three times a day) were required to maintain the ketosteroid output below 8 mg. per day.

cortisone on 2 sisters, J. S. and M. C. S. The intramuscular administration of 50 mg. of cortisone daily decreased the output of 17-ketosteroids to an average level of 4.5 mg. per day in the case of J. S., whereas 50 mg. of cortisone given daily by mouth decreased the ketosteroids to an average of 21 mg. per day in the case of M. C. S. When the same dose was given to this patient by the intramuscular route, the 17-ketosteroids fell to a level of 8 mg. per day after seven days.

Figure 4 shows a comparison of the intramuscular and the oral administration of cortisone in the case of W. D. In March 1950, the intramuscular administration of 25 mg. of cortisone per day caused a gradual decrease in the output of 17-ketosteroids from an average of 48 mg. per day to less than 8 mg. per day. This decline in the excretion was interrupted by

a temporary increase during a slight respiratory infection. In February 1951, the oral administration of 25 mg. of cortisone reduced the ketosteroids from an average of 30 mg. per day to a level between 17 and 31 mg., with an average of 22 mg. Increasing the dose to 37.5 mg. (12.5 mg. three times a day) resulted in a further reduction of the output to an average of 13 mg. per day. This period was interrupted by a slight cold, which again caused a rebound of the 17-ketosteroid excretion similar to that which had occurred during the study with intramuscular cortisone. It was

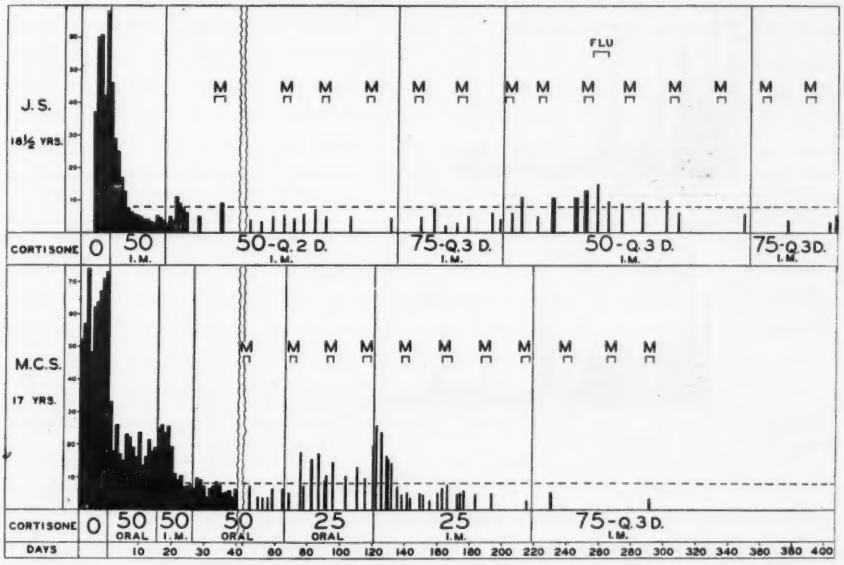


FIG. 3. Comparison of the effects of intramuscular and oral cortisone on the suppression of urinary 17-ketosteroids of 2 sisters who were female pseudohermaphrodites. ("M" represents menstrual periods.)

necessary to increase the dose of oral cortisone to 50 mg. per day in order to bring about suppression of the 17-ketosteroids below the level of 8 mg. per day.

*Rapidity of action of oral vs. intramuscular cortisone*

The suppression of ketosteroid excretion occurred more rapidly with the oral than with the intramuscular administration of cortisone. Figures 2 3 and 4 show that when the drug was given intramuscularly there was a gradual decline in the output, usually beginning on the second day and progressing for six or seven days. In the case of M. C. S. (Fig. 3), the change from oral to intramuscular cortisone was not manifested until the sixth

day. In contrast, the oral administration of cortisone usually caused a marked decrease of the urinary ketosteroids on the first day.

*Duration of effect of oral vs. intramuscular cortisone*

Figure 2 (P. R.) shows that when intramuscular injection of 100 mg. of cortisone was discontinued, a period of ten days elapsed before the ketosteroid excretion began to increase. On the other hand, when the dose of oral cortisone was decreased from 100 mg. to 25 mg. per day, there

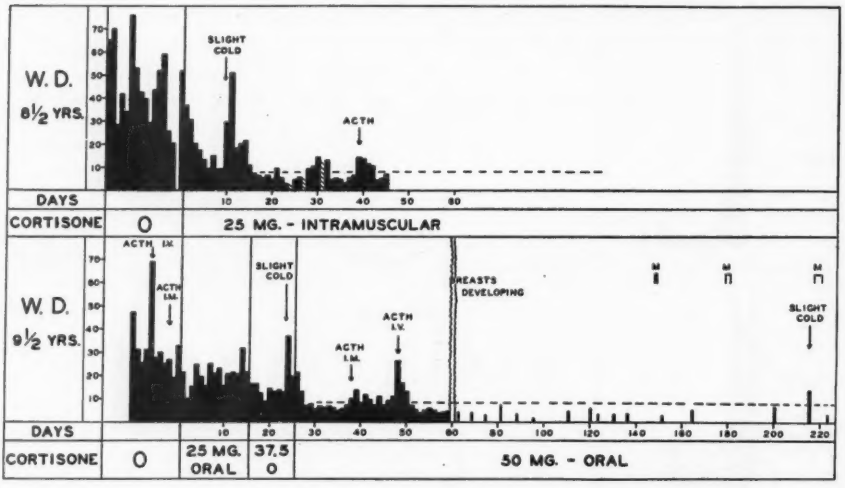


FIG. 4. Comparison of the effects of intramuscular and oral cortisone on the suppression of urinary 17-ketosteroids of a female pseudohermaphrodite.

Note the temporary increases in ketosteroid excretion which occurred during slight colds and the relatively smaller responses to test doses of intramuscular or intravenous ACTH given over periods of twenty-four or twelve hours. ("M" represents menstrual periods.)

was an immediate increase in the excretion. Figure 3 (M. C. S.) shows also that when treatment was changed from 25 mg. of cortisone given orally to 25 mg. by injection, there was a sudden increase of ketosteroid excretion due to the fact that there was a rapid release from the suppressive effects of oral cortisone before the slower action of intramuscular cortisone became effective. Figure 6 (L. U.) shows the effects in a young infant of abruptly discontinuing the oral administration of 5 mg. of cortisone daily on the 227th and again on the 280th day of treatment. In each instance there was an immediate rise in the output of ketosteroids.

*Frequency of oral medication*

Because of the relatively rapid and evanescent effects of oral cortisone, the patients were given oral medication in 2, 3 or 4 equally divided doses

each day. We observed no definite difference in these methods, but perhaps this point should be studied more intensively. In the case of M. C. S., a change from 25 mg. twice a day to 12.5 mg. four times a day caused no definitely increased effect.

*Maintenance dose of oral vs. intramuscular cortisone*

Table 1 shows the doses of *intramuscular* cortisone required to maintain suppression of the ketosteroids below the level of 8 mg. per day. In the case of J. S. (Fig. 3), suppression to this degree was maintained by the intramuscular administration of 50 mg. of cortisone every second day and by 75 mg. every third day.[1] When the dose was decreased to 50 mg. every third day, the urinary 17-ketosteroids rose above this level. In the case of M. C. S., 25 mg. of cortisone (12.5 mg. twice a day)given orally failed to maintain adequate suppression, but the intramuscular administration of 25 mg. daily or 75 mg. every third day was effective. In the case of M. B. (Table 1), the same dose of intramuscular cortisone was required, 50 mg. every third day being inadequate and 75 mg. every third day being effective. In summary, as shown in the Table, in the group of older patients the maintenance dose of 25 mg. of cortisone per day given *intramuscularly* suppressed the output of 17-ketosteroids to levels of from 4 to 6 mg. per day, but a decrease to 16.6 mg. daily permitted the output to increase to an average of 9.6 mg. per day, with occasional rises to much higher values. Equal effects were obtained by giving 25 mg. each day, 50 mg. every second day or 75 mg. every third day.[1]

Figures 2, 3, 4 and 5 and Table 1 show the doses of *oral* cortisone required to maintain the same suppression of 17-ketosteroid excretion. In the case of P. R. (Fig. 2), the administration of 50 mg. of cortisone orally did not maintain uniform suppression of the ketosteroids. It is of interest that during a period of 130 days while this dose was given, although the patient's breasts developed, there was no definite change in the growth of facial hair or in her acne. After increasing the dose of oral cortisone to 75 mg. daily (25 mg. three times a day), the urinary 17-ketosteroids remained below the level of 8 mg. per day (average, 5 mg. per day) and the evidences of virilization decreased. In the case of M. C. S. (Fig. 3), the initial treatment with 50 mg. of oral cortisone did not reduce the urinary 17-ketosteroids below the level of 21 mg. per day. However, after suppression was obtained with 50 mg. of intramuscular cortisone, the same amount of oral cortisone seemed adequate to maintain suppression during the next thirty-six days. During this period, increasing weight and fullness of the face suggested that this amount of cortisone might exceed the

[1] We have subsequently shown that in the case of J. S. the intramuscular administration of 100 mg. of cortisone every fourth day is equally effective.

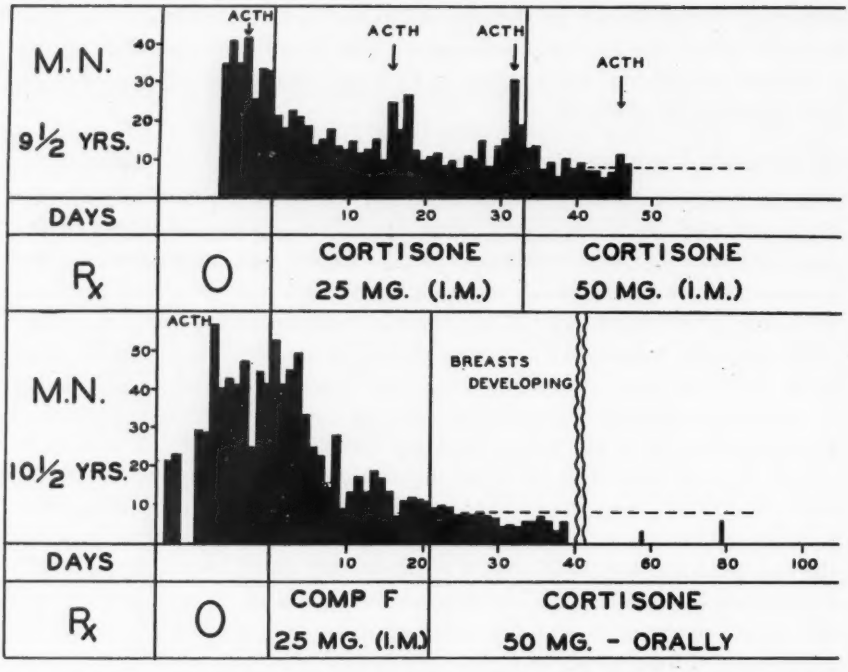


FIG. 5. Comparison of the effects of intramuscular compound F and intramuscular and oral cortisone on the suppression of urinary ketosteroids of a female pseudohermaphrodite.

patient's metabolic requirements. When the dose of oral cortisone was decreased to 25 mg. per day, the output of 17-ketosteroids rose to an average level of 13.4 mg. per day. Accordingly, the administration of 25 mg. of cortisone was changed to the intramuscular route. In the case of W. D. (Fig. 4), a dose of 50 mg. of oral cortisone daily has been adequate to maintain full adrenal suppression. The same dose has been adequate in the case of M. N. (Fig. 5). In short, when cortisone is given *orally* instead of intramuscularly, it is more difficult to maintain uniform suppression of the urinary ketosteroids and a larger dose is required—approximately 50 or 75 mg. per day as compared to 25 mg. of intramuscular cortisone.

### DOSAGE OF INTRAMUSCULAR AND ORAL CORTISONE IN YOUNG INFANTS (GROUP B)

Treatment with cortisone was begun in 3 female pseudohermaphrodites between the ages of 11 and 19 weeks (Figs. 6 and 7). These patients all presented serious symptoms of defective electrolyte regulation and

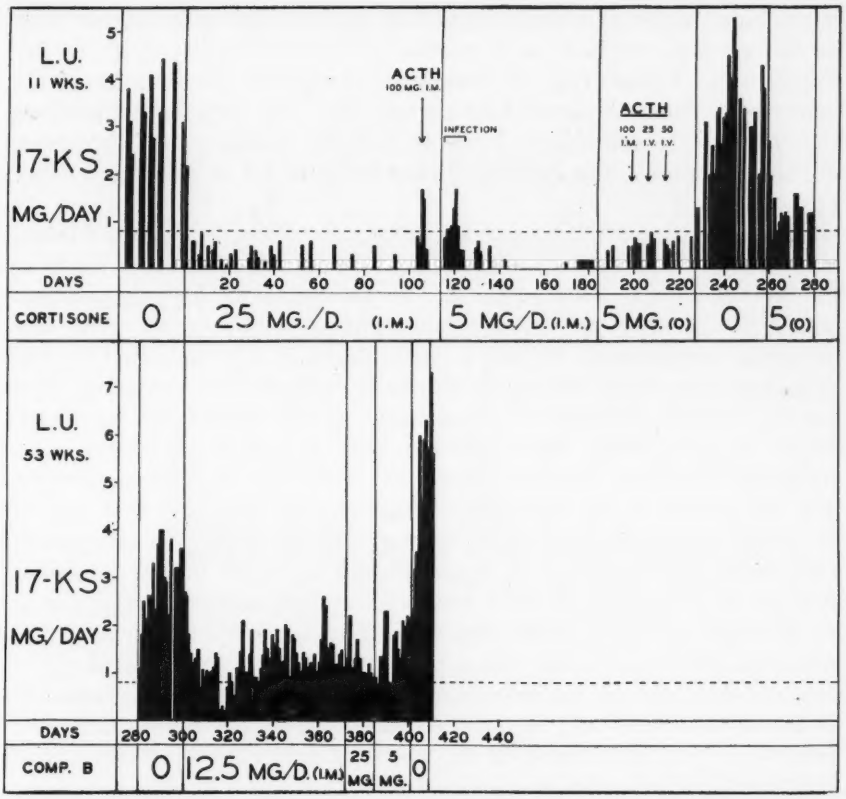


FIG. 6. Effects of intramuscular and oral cortisone and of compound B given intramuscularly on the ketosteroid excretion of an infant female pseudohermaphrodite with marked tendency to sodium loss.

Compound B seemed to have less effect on the suppression of ketosteroid excretion than did cortisone but had more influence on sodium loss.

Note that although large doses of intramuscular and intravenous ACTH caused no increase in the output of 17-ketosteroids while the patient was receiving 5 mg. of oral cortisone, there was a prompt rise of urinary 17-ketosteroids when treatment was omitted.

required additional treatment with a high NaCl intake and/or desoxycorticosterone. The influence of cortisone on electrolyte regulation will be discussed in a subsequent paper. In these young infants it was by no means certain what level of urinary ketosteroid excretion was indicative of optimal suppression of adrenal activity. During the first few years of life the normal output of 17-ketosteroids is usually less than 0.5 mg. per day, although it sometimes rises as high as 1.0 mg. per day. It must be

remembered also that a small portion of the cortisone administered may be converted in the body and excreted as 17-ketosteroids (3, 4). In the infants under 1 year of age we attempted to suppress the excretion of 17-ketosteroids below the level of 0.8 mg. per day. This value is indicated on the charts by a dotted line. In recent months, however, we have been unable to maintain the excretion below levels of 1.1 to 1.7 mg. per day (Table 1).

L. U. (Fig. 6) was first treated with 25 mg. of cortisone daily given intramuscularly. This caused a prompt decrease in urinary 17-ketosteroids from an average level of 3.5 mg. per day to an average of 0.4 mg. per day. Although this dose of cortisone was believed to be excessive for the metabolic requirements of such a small infant, it was continued for 116 days in order to study thoroughly the effects on electrolyte regulation. During this time the infant became obese and a round "moon face" developed, which was not florid. Linear growth and epiphyseal ossification were inhibited. The dose of intramuscular cortisone was then decreased to 5 mg. daily and on the 186th day treatment was changed to 5 mg. of cortisone daily by the oral route, 2.5 mg. suspended in syrup being given twice a day. These changes in treatment did not result in any significant increase in urinary ketosteroids except for a slight temporary rise during an infection about the 120th day. During treatment with 5 mg. of oral cortisone daily, tests with 100 mg. of ACTH given intramuscularly in four divided doses at six-hour intervals, and with intravenous infusions of 25 mg. and of 50 mg. of ACTH each given by continuous drip over twelve-hour periods, caused no rise in ketosteroids. On the 227th day of treatment cortisone was discontinued and there was a prompt rise of the urinary ketosteroids to the previous level of from 3 to 5 mg. per day. Subsequently the effects of compound B (corticosterone) were studied in this patient and will be discussed later. After discontinuing compound B, the 17-ketosteroid output rose to about 6 mg. per day. Subsequently treatment with 12 mg. of oral cortisone daily (4 mg. three times a day) has succeeded in reducing the ketosteroids to only 1.7 mg. per day.

Patient F. F. (Fig. 7) was treated with 25 mg. of oral cortisone in divided doses for twenty-seven days. In the latter part of this period the urinary 17-ketosteroids, which before treatment ranged from 3.5 to 6.2 mg. per day, with an average of 4.3 mg. per day, fell to a level of 1.4 mg. per day. It was thought that possibly a portion of the ketosteroids excreted might be derived from the cortisone administered. The dose of oral cortisone was decreased to 12 mg. per day (6 mg. twice a day). Since then the average output of ketosteroids has been 1.1 mg. per day but there have been some fluctuations. This dose has been continued until the present.

S. K. (Fig. 7) was treated initially with 12.5 mg. of cortisone given

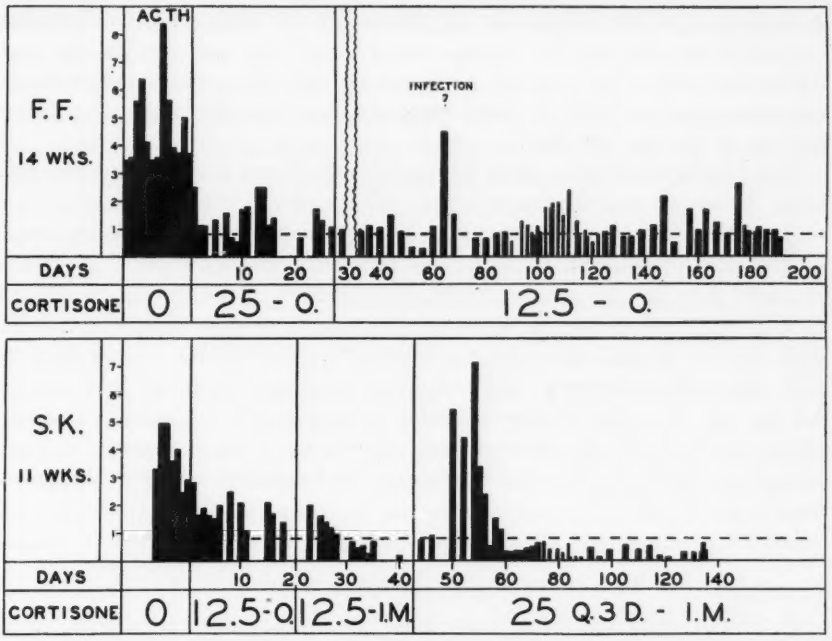


FIG. 7. Effects of oral and intramuscular cortisone on the excretion of 17-ketosteroids of 2 infant female pseudohermaphrodites with marked tendency to sodium loss.

S. K. has subsequently been treated with 25 mg. of intramuscular cortisone every fourth day and the urinary ketosteroids now average 1.3 mg. per day.

Note the marked temporary increase of ketosteroid output which occurred during an illness of unknown origin.

orally. The urinary 17-ketosteroids were reduced from an average of 3.2 mg. per day to an average of 1.7 mg. per day, which we believed did not indicate full suppression of adrenal activity. On the twentieth day treatment was changed to 12.5 mg. of cortisone given by the intramuscular route, with a reduction of the ketosteroid output to 0.5 mg. per day. When the dose of intramuscular cortisone was decreased still further on the forty-third day to 25 mg. every third day (an average of 8.3 mg. per day), the ketosteroid excretion remained suppressed to an average of 0.3 mg. per day, except for a temporary escape to levels of from 4 to 7 mg. per day between the fiftieth and fifty-fifth days of treatment. The cause of this rise, which subsided without change in the dose of cortisone, is not certain. The infant seemed sick, although no apparent infection was found. As will be reported later, the temporary rise in ketosteroids was accompanied by a marked fall in the level of serum sodium and rise in the level of serum

potassium. On the 123rd day of treatment the dose of intramuscular cortisone was decreased to 25 mg. every fourth day and the patient was discharged from the hospital. Although at first the urinary ketosteroids remained at a low level, a recent recheck shows that they have risen to an average of 1.3 mg. per day.

The limited studies in these young infants do not permit exact conclusions either concerning the minimal suppressive doses of intramuscular and oral cortisone, or concerning the degree to which the ketosteroid output can be reduced. It is obvious that considerably smaller doses are required than in the older patients. At present we are treating L. U. and F. F. with 12 mg. of *oral* cortisone daily (4 mg. three times a day) and S. K. with 25 mg. of *intramuscular* cortisone every fourth day (equivalent to 6.25 mg. daily). Although the outputs of ketosteroids range from 1.1 to 1.7 mg. per day, there is no evidence of progressive virilization and the infants apparently are growing and developing at normal rates. Longer periods of observation will be required to determine whether the doses employed are optimal.

### TREATMENT WITH CORTISONE OF 2 BOYS, AGED 7 YEARS AND 4½ YEARS (GROUP C)

T. T. (Table 1) was a boy of 6 years, 10 months, who had macrogenitosomia praecox, with a height age of 9½ years and a bone age of 13½ years. Before treatment the average output of 17-ketosteroids was 26 mg. per day. Daily treatment with 100 mg. of cortisone given intramuscularly resulted in a decr e of urinary ketosteroids to 3.8 mg. per day. On the eighth day the dose was changed to 50 mg. of intramuscular cortisone given every second day, and on the thirty-fourth day to 75 mg. every third day. Treatment has now been continued for six months, with maintenance of the ketosteroids at an average of 3.5 mg. per day and without evidence of overdosage.

The case of W. J. (Table 1) was more difficult to interpret. In February 1950, when treatment with cortisone was first tried, this boy was 2½ years old and had macrogenitosomia praecox, with an output of 17-ketosteroids averaging 8.6 mg. per day. In addition, he had marked hypertension causing cardiac decompensation. The intramuscular administration of 50 mg. of cortisone daily for eleven days caused a decrease of the 17-ketosteroids to 1.4 mg. per day, but the patient became excited and manic and the blood pressure rose to such an extent that cortisone was discontinued. Following this, the ketosteroid excretion increased again until it reached 11 mg. per day after three weeks. Cortisone therapy was not tried again until December 22, 1950. In the meantime the patient's condition deteriorated progressively, with persistent hypertension and in-

creasing cardiac failure in spite of digitalis therapy. There were repeated respiratory infections and attacks of tonsillitis. The ketosteroid excretion showed a tendency to fall, possibly as a result of his generally poor health. There were also evidences of impaired glycogenetic function of the adrenal. By December 1950, evidences of cardiac failure were pronounced; the blood pressure fell to a low level, and death seemed imminent. The ketosteroid output at this time was between 1.2 and 3.0 mg. per day. Cortisone therapy was tried once again on a somewhat empirical basis, but doses of 12.5 mg. per day were given intramuscularly, whereas 50 mg. a day had been used previously. There was a spectacular improvement in the cardiac condition. The blood pressure rose to normal levels, but hypertension did not recur. In June 1951, treatment was changed to 25 mg. of oral cortisone daily. Since cortisone was begun, the 17-ketosteroid excretion has remained between 0.5 and 2.4 mg. per day, with an average of 1.3 mg. per day. One cannot be sure that this was due to the suppressive effect of cortisone, because the ketosteroid excretion was already at a comparatively low level before treatment was begun. The effects of cortisone on hypertension in this and other cases of congenital adrenal hyperplasia will be discussed in a subsequent paper.

### EFFECTS OF COMPOUND F AND COMPOUND B ON THE SUPPRESSION OF KETOSTEROID EXCRETION

*Compound F* (*17-hydroxycorticosterone*) was studied in the case of M. N. (Fig. 5). A year previously, 25 mg. of intramuscular cortisone daily had reduced the 17-ketosteroid excretion from a level of 28 mg. to an average of 11.8 mg. per day. Increasing the dose of intramuscular cortisone to 50 mg. per day caused a further decrease in the ketosteroid output to an average of 7.1 mg. per day. The administration of 25 mg. of compound F daily by the intramuscular route caused the 17-ketosteroid excretion to decrease from an average of 34 mg. per day to levels of 7 to 17 mg. per day, with an average of 12 mg. per day. This effect was approximately the same as that obtained with an equal dose of cortisone. No changes in carbohydrate metabolism or other evidences of overdosage were observed. Unfortunately the patient could not be kept in the hospital long enough to try the effects of other dose levels of compound F. Prior to the patient's discharge from the hospital, treatment was changed to 50 mg. of oral cortisone daily. This has maintained the ketosteroids at an average level of 5 mg. per day.

*Compound B* (*corticosterone*) was tried in the case of L. U., a young female pseudohermaphrodite with defective electrolyte regulation (Fig. 6). Since the age of 11 weeks this patient had received cortisone continuously over a period of 227 days. Following a period of withdrawal of therapy

for thirty-two days, cortisone was given again for twenty-one days. Prior to treatment with compound B, all hormonal treatment was discontinued again for twenty-one days, during which the ketosteroid excretion rose to levels of 2.1 to 4.0 mg. per day, with an average of 2.9 mg. per day. She was then given intramuscular injections of 12.5 mg. of compound B daily. Within four days there was a decrease of the ketosteroid output to an average of 1.3 mg. per day. This degree of suppression was less than that obtained originally with 25 mg. of intramuscular cortisone and subsequently maintained by 5 mg. of intramuscular cortisone or 5 mg. of cortisone given orally. After seventy-two days of treatment with compound B, the dose was increased to 25 mg. daily given intramuscularly. This was continued for twelve days and at the end of this time the urinary ketosteroids had fallen to 1.0 mg. per day. When the dose of compound B was reduced to 5 mg. daily, the output of ketosteroids increased to 1.8 mg. per day.

During treatment with compound B, studies of the serum electrolytes were made both when the patient was receiving a high NaCl intake and when no salt was added to her diet. A comparison with similar observations made during cortisone therapy indicated that compound B had considerably more effect on sodium retention than did cortisone. These studies will be reported in another paper.

### IS THE ADRENAL PERMANENTLY DAMAGED BY CORTISONE?

Considerably more time and study are required before one can determine the ultimate effect of long-continued treatment with cortisone upon the adrenal activity of patients with congenital adrenal hyperplasia. It is apparent from our studies that even when the endogenous activity of the adrenals, as measured by the output of 17-ketosteroids, has been suppressed by cortisone, the glands are capable of responding to some stresses induced by infection or other causes. In the case of W. D. (Fig. 4), slight upper respiratory infections occurring in the early stages of intramuscular cortisone therapy and again during oral treatment with cortisone caused marked but temporary increases in the output of 17-ketosteroids. Among the infants treated (Figs. 6 and 7), temporary increases in the 17-ketosteroid excretion occurred in L. U. on the 120th day of cortisone therapy, in F. F. on the sixty-second day and in S. K. on the fiftieth day. At these times the infants seemed sick. L. U. and F. F. had mild upper respiratory infections with slight rises of temperature. S. K. appeared ill, but had no fever or any evidence of infection. These periods of increased ketosteroid excretion were accompanied by an increased tendency to lose sodium. This was especially marked in the case of S. K., in whom the serum sodium

fell from 135 to 125 mEq./L. and the serum potassium rose from 5.8 to 6.8 mEq./L., both returning to previous levels after the urinary ketosteroids decreased without change of therapy.

Attempts were made in the cases of W. D., M. N. and L. U. (Figs. 4, 5 and 6) to test the responsiveness of the adrenals by administering ACTH. Before treatment, W. D. showed a marked increase in the excretion of 17-ketosteroids during one day when she was given 50 mg. of ACTH by continuous intravenous drip over a period of twelve hours. On the same day there was a marked increase of urinary sodium. When tested four days later with 100 mg. of ACTH given intramuscularly (25 mg. every six hours), there was no response. After thirty-eight days of cortisone therapy there was no response to the same dose of intramuscular ACTH, but on the forty-eighth day there was a definite response to the intravenous administration of ACTH. This response, however, was smaller than that obtained before cortisone therapy.

The case of L. U. (Fig. 6) is of special interest. Unfortunately it was not considered safe to test this patient with ACTH before cortisone therapy, because of her severe electrolyte disturbance. Between the 198th and 216th days of therapy, at a time when she was receiving only 5 mg. of oral cortisone daily, tests with 100 mg. of intramuscular ACTH (25 mg. every six hours for four doses), and 25 and 50 mg. of ACTH given by continuous intravenous drip over a period of twelve hours failed to cause any response in the excretion of 17-ketosteroids. This suggested that the adrenal had been permanently damaged. This impression, however, was refuted by the fact that withdrawal of cortisone therapy on the 227th day caused an immediate increase in the ketosteroid excretion to the pre-treatment level. Subsequently, when cortisone therapy was discontinued on the 280th day, the ketosteroid excretion rose to a level of from 3 to 4 mg. per day and when Compound B was discontinued on the 400th day, it increased to between 6 and 7 mg. per day. The fact that under cortisone therapy there was a complete absence of response to relatively large doses of ACTH (administered for twelve or twenty-four hours only) suggested that cortisone is capable of blocking the action of ACTH on the adrenal and that it does not merely inhibit the secretion of ACTH by the pituitary, as we had stated in a previous publication (3).

It should be noted that the increases of 17-ketosteroids which occurred under the stimulation of infections or other stresses in the cases of L. U. (Fig. 6), F. F. and S. K. (Fig. 7), W. D. (Fig. 4) and M. N. (Fig. 5), were considerably greater than those which occurred with the doses of ACTH administered. This may indicate that such stresses lead to the secretion by the pituitary of larger amounts of ACTH than were given as test doses.

Another explanation, however, may be that the stresses of infections probably result in increased secretion of ACTH over a number of days, whereas the tests with ACTH were of only twelve or twenty-four hours' duration.

## SUMMARY AND CONCLUSIONS

This report is based on observations made on 11 patients with congenital adrenal hyperplasia treated with cortisone continuously for periods of from five to seventeen months. It would be unwise from these limited studies to draw conclusions concerning the optimal method of treatment. Our findings are published merely as a guide for others who desire to treat such cases.

To prevent progressive virilization and obtain the best clinical results it is probably desirable to suppress as completely as possible the abnormal androgenic activity of the patient's adrenals. Since therapy will probably have to be continued for a long period and possibly throughout life, it is obviously desirable to use the smallest dose of cortisone which will maintain adequate suppression of adrenal activity, in order to avoid the dangers of excessive metabolic or toxic effects, and also to save expense. The minimal maintenance dose must be determined for each patient, using the urinary excretion of 17-ketosteroids and the clinical symptomatology as guides. In patients 8 years of age or older, we believe that the excretion of 17-ketosteroids should be suppressed at least to the level of 8 mg. per day, as we have seen a tendency to the return of virilization when the excretion has risen higher. With the doses we have employed, it does not seem possible to decrease the output of ketosteroids below a level of from 3 to 6 mg. per day in the older group. In younger patients it is not certain what level of ketosteroid excretion is indicative of optimal suppression of the androgenic activity of the adrenals. Normal infants under 1 year of age excrete less than 0.5 mg. of 17-ketosteroids per day. Although these low levels have been attained at times, it is difficult to maintain the output below 1.0 or 1.7 mg. per day. Perhaps these amounts represent metabolic products of the administered cortisone. In children between the ages of 1 and 8 years, the degree to which the urinary ketosteroids can be suppressed will have to be determined by trial.

On the basis of our experience, we feel justified in making the following tentative suggestions:

1. To obtain rapid suppression of adrenal activity, *initial treatment* with relatively large doses of intramuscular cortisone may be given. For patients 8 years of age or over, 50 mg. of intramuscular cortisone per day are adequate; in infants, 25 mg. per day. We prefer to begin treatment with intramuscular rather than oral cortisone, even though it acts less

rapidly, because the results are more predictable. The maximum suppression of urinary ketosteroids should be attained within five to ten days, after which the maintenance dose should be determined.

2. For patients over 8 years of age the *maintenance dose* of cortisone given *intramuscularly* is usually 25 mg. per day. Treatment is just as effective when spaced at longer intervals of two, three or four days. For example, 75 mg. of cortisone may be given every third day, or 100 mg. every fourth day. Perhaps even longer intervals may prove satisfactory.

When cortisone is given *orally*, considerably larger doses are required. In the cases we have treated, 50 or 75 mg. per day have been needed. These amounts should be given in equally divided doses, two, three or four times a day.

3. For young infants, the maintenance dose of cortisone is smaller. In our patients, *intramuscular* injections of 5 mg. daily and of 25 mg. every fourth day (equivalent to 6.25 mg. per day) were effective. By the *oral* route, 5 mg. and 12.5 mg. per day seemed to maintain suppression, but larger amounts may be required in some cases. For children between the ages of 1 and 8 years, the maintenance dosage is probably somewhere between these amounts and 25 mg. per day, when the drug is given intramuscularly. It should be determined for each individual.

4. These observations suggest that for its suppressive action, two or three times as much cortisone is required when it is given orally as when injected intramuscularly. Other workers have differed concerning the comparative effectiveness of intramuscular *vs.* oral cortisone. Boland and Headley (5) studied 14 patients with rheumatoid arthritis in whom cortisone therapy was changed from the intramuscular to the oral route. Eight of these patients, when given oral cortisone, required 1.25 to 1.3 times the intramuscular dose to produce adequate response. Kuzell and Schaffarzick (6) found the figure to be 1.25 to 3.0 in a variety of diseases. In our experience, intramuscular cortisone has the advantage of being only one half to one third as expensive, because smaller amounts are required. The disadvantage of an intramuscular injection every third or fourth day (which can be given by the parent or the patient) is scarcely greater than the annoyance of administering oral medication from two to four times a day. The effects of intramuscular medication seem to us more predictable, probably because of the more uniform rate of absorption; moreover, some of the cortisone may be lost when it is given to infants in a syrup.

5. In young infants who have the sodium-losing type of congenital adrenal hyperplasia, it is important that treatment include a high sodium intake and/or desoxycorticosterone in addition to cortisone. This will be discussed in a subsequent paper.

6. While patients are receiving cortisone therapy, there are at times periods of temporarily increased output of 17-ketosteroids due to the stress of infections or some other cause. These episodes are especially common in young infants and may be accompanied by a dangerous loss of sodium if there is a defect of electrolyte regulation. The dose of cortisone should not be altered during these periods, but increased administration of NaCl or desoxycorticosterone may be indicated.

7. In the 1 case studied, compound F in doses of 25 mg. per day seemed to have approximately the same effect as cortisone on the suppression of adrenal activity.

8. In an infant with the salt-losing type of adrenal hyperplasia, compound B seemed to have somewhat less suppressive action than cortisone, but a definite sodium-retaining effect was exerted by doses of 12.5 mg. per day.

9. The ultimate effects on the adrenal of the amounts of cortisone administered are not known. Although cortisone seemed to suppress the response of the adrenal to 24-hour tests with ACTH, the activity of the adrenals as measured by the 17-ketosteroid excretion returned promptly when cortisone was discontinued after 227 days of continuous therapy.

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# FURTHER STUDIES ON THE TREATMENT OF CONGENITAL ADRENAL HYPERPLASIA WITH CORTISONE

## II. THE EFFECTS OF CORTISONE ON SEXUAL AND SOMATIC DEVELOPMENT, WITH AN HYPOTHESIS CONCERNING THE MECHANISM OF FEMINIZATION*†

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IN a previous paper (1) we have reported the results of cortisone therapy in a series of 8 patients with congenital adrenal hyperplasia, who were treated for periods varying from ten days to five months. Two of these patients have now been treated continuously for seventeen months, and 1 for fifteen months. Four of the series, who were treated for relatively short periods, and 4 new patients have subsequently begun continuous therapy. The present report deals with these 11 patients who now have received cortisone continuously for periods of from five to seventeen months. Studies comparing the minimal doses of oral and of intramuscular cortisone necessary to maintain adrenal suppression, as determined by the excretion of 17-ketosteroids, are presented in another communication (2). This paper is concerned with the clinical effects of cortisone therapy, particularly the effects on sexual and somatic development. For the purpose of discussion, the patients are divided on the basis of their age and sex into three groups.

### GROUP A—OLDER FEMALE PSEUDOHERMAPHRODITES

Six female pseudohermaphrodites who were between the ages of $8\frac{1}{2}$ and

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TABLE 1. GROUP A—FEMALE PSEUDOHERMAPHRODITES OVER 8 YEARS OF AGE

| Before treatment | | | | | | | Treatment with cortisone | | | | | Results of treatment | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Name | Age (yrs.) | Height age | Bone age | Wt. (Kg.) | 17-KS, mg./day | Associated symptoms | Date begun | Route | Average daily dose (mg.) | How given | Duration (mos.) | 17-KS, mg./day | Breast development | Estrin. vag. smear | Menstruation | Decreased hirsutism | Decreased acne | Growth (ins.) | Weight change (Kg.) | Other effects |
| M.B. | 8½ | 13 | 17 | 40 | 38 | | 6- 5-50 | i.m. | 100 | 100 q d | 17 | 5.7 | ++ | ++ | 0 | + | + | 0.5 | +1 | |
| | | | | | | | 6-11-50 | i.m. | 25 | 25 q d | | 5.3 | | | | | | | | |
| | | | | | | | 10- 8-50 | i.m. | 25 | 50 q 2d | | 5.7 | | | | | | | | |
| | | | | | | | 2-27-51 | i.m. | 16.6 | 50 q 3d | | 9.6 | | | | | | | | |
| | | | | | | | 5-16-51 | i.m. | 25 | 75 q 3d | | 6.2 | | | | | | | | |
| | | | | | | | 10- 7-51 | i.m. | 25 | 100 q 4d | | 7.4 | | | | | | | | |
| J.S. | 18½ | 13 | Fused | 50 | 53 | Hypertension; pigmentation | 6-19-50 | i.m. | 50 | 50 q d | 17 | 4.5 | ++ | +++ | +++ | +++ | +++ | 0 | 0 | Decreased B.P. Depigmentation |
| | | | | | | | 7-17-50 | i.m. | 25 | 50 q 2d | | 5.0 | | | | | | | | |
| | | | | | | | 11- 4-50 | i.m. | 25 | 75 q 3d | | 4.8 | | | | | | | | |
| | | | | | | | 1- 6-51 | i.m. | 16.6 | 50 q 3d | | 9.6 | | | | | | | | |
| | | | | | | | 6-11-51 | i.m. | 25 | 75 q 3d | | 3.8 | | | | | | | | |
| | | | | | | | 8-28-51 | i.m. | 25 | 100 q 4d | | 5.6 | | | | | | | | |
| M.C.S. | 16½ | 13½ | Fused | 51 | 62 | Hypertension; pigmentation | 11- 4-50 | 0 | 50 | 25×2 | 12 | 21.0 | ++ | +++ | +++ | +++ | +++ | 0.4 | −1 | Decreased B.P. Depigmentation |
| | | | | | | | 11-14-50 | 0 | 50 | 12.5×4 | | 17.0 | | | | | | | | |
| | | | | | | | 11-19-50 | i.m. | 50 | 50 q d | | 8.0 | | | | | | | | |
| | | | | | | | 11-30-50 | 0 | 50 | 25×2 | | 6.0 | | | | | | | | |
| | | | | | | | 1- 9-51 | 0 | 25 | 12.5×2 | | 13.4 | | | | | | | | |
| | | | | | | | 3- 4-51 | i.m. | 25 | 25 q d | | 4.8 | | | | | | | | |
| | | | | | | | 6-11-51 | i.m. | 25 | 75 q 3d | | 5.0 | | | | | | | | |
| P.R. | 16 | 13½ | Fused | 66 | 50 | | 11-12-50 | 0 | 100 | 50×2 | 12 | 7.0 | ++ | + | 0 | + | + | 0.5 | +2.5 | |
| | | | | | | | 11-28-50 | 0 | 25 | 12.5×2 | | 19.0 | | | | | | | | |
| | | | | | | | 12- 5-50 | 0 | 50 | 25×2 | | 13.0 | | | | | | | | |
| | | | | | | | 4- 6-51 | 0 | 75 | 25×3 | | 5.0 | | | | | | | | |
| W.D. | 9½ | 13½ | 15 | 41 | 30 | | 2- 3-51 | 0 | 25 | 12.5×2 | 9 | 22.0 | ++ | +++ | ++ | + | ++ | 0 | +6.6 | |
| | | | | | | | 2-18-51 | 0 | 37.5 | 12.5×2 | | 13.0 | | | | | | | | |
| | | | | | | | 2-28-51 | 0 | 50 | 25×2 | | 4.0 | | | | | | | | |
| M.N. | 10½ | 14½ | 16 | 39 | 34 | | 5-25-51 | Comp. F, 25 i.m. q d | | | 5 | 12.0 | + | + | 0 | ? | ++ | 0 | +1.5 | |
| | | | | | | | 6-15-51 | Oral cortisone, 50 (25×2) | | | | 5.0 | | | | | | | | |

18½ years when therapy was begun have now been treated with cortisone for periods of from five to seventeen months. The type and duration of treatment are shown in Table 1.

During the period of study none of these patients received any hormone except cortisone. Prior to treatment 2 patients had received female sex hormones. P. R. had been given stilbestrol in small doses in 1942 at the age of 7½ years. In June 1943 continuous treatment was begun with 1 mg. of stilbestrol daily. Between September 1943 and March 1944 she received 2 mg. of stilbestrol and 20 mg. of anhydrohydroxyprogesterone daily. Then anhydrohydroxyprogesterone was discontinued, but stilbestrol was continued until February 1945. After that time she received no hormonal medication. Under treatment with estrogen and progestin the breasts had developed, but hirsutism and acne had increased and the urinary 17-ketosteroids had not decreased. At the time of treatment with cortisone her breasts were somewhat enlarged and pendulous but seemed to consist entirely of fatty tissue. The vaginal smear showed no evidence of estrogenic effects. J. S. had been treated with stilbestrol between 1941 and 1943 when she was 9 to 11 years of age. The breasts had developed slightly and scanty menstrual spotting occurred on two occasions. After discontinuing stilbestrol the breasts disappeared entirely and no further menstrual bleeding occurred. There were no estrogenic manifestations at the time cortisone was begun.

*Breast development.* With the exception of P. R., who had fatty deposits in the mammary region, none of the girls had any breast, nipple or areolar development at the time of beginning cortisone treatment. Definite breast development occurred in every case. In most instances a small nodule of glandular tissue became palpable within two or three weeks after the urinary 17-ketosteroids had been reduced to a level of 8 mg. per day or less. The development of the breasts, nipples, and areolae proceeded as it does in normal adolescent girls. In all patients except P. R. the breasts retained the characteristics of adolescence; the maximum diameter of glandular tissue attained was between 7 and 9 cm. The progress of development seemed much more rapid than that which occurs in normal puberty. P. R.'s breasts became firmer, more nodular and pendulous, the fatty tissue apparently becoming infiltrated with glandular elements. In no instance was there hyperpigmentation of the areolae such as occurs with stilbestrol therapy.

*Vulvar development and vaginal smears.* Concomitant with the mammary development, the mucous membranes of the vulva lost the bright, pink, smooth appearance which is present before puberty and became thickened, paler and covered with increased secretions. The growth of the labia minora varied in different patients, depending upon the extent to which the rudi-

ments had developed in embryonic life. In some pseudohermaphrodites there is practically no embryonic development of labia minora. In several of our patients, short but thick corrugated, pigmented fleshy folds representing the labia minora developed during cortisone therapy. In others there were only small rudimentary folds. The vaginal smears, which were entirely of the infantile type before treatment, showed rapidly the changes characteristic of adolescence. The cells became larger, polygonal, with small pyknotic nuclei and some of them stained red with Shorr's stain.

*Menstruation.* To date, menstruation has occurred in 3 cases. J. S., aged 18½ years, menstruated on the thirty-third day after cortisone therapy was begun, or twenty-eight days after the urinary ketosteroids were reduced to a minimal level. Since then she has menstruated at intervals of from twenty-eight to thirty-one days and has now had a total of 17 periods. The flow has been of normal amount and not accompanied by cramps. Her sister, M. C. S., aged 16¼ years, menstruated on the forty-second day after starting cortisone. This was the twenty-third day after the ketosteroids were reduced below the level of 8 mg. per day. Her periods recurred at intervals varying from twenty-two to twenty-eight days. On a number of occasions she had menstrual cramps. There have now been a total of 14 periods. In the case of W. D. cortisone treatment was begun at the age of 9½ years, at which time the patient's height-age was 13½ years and bone-age 15 years. She first menstruated five months after beginning therapy, or three and one half months after the ketosteroid excretion had been suppressed. She has now had 4 periods at intervals of from fourteen to thirty-nine days. Although the orifice of the urogenital sinus is quite small, she has had no cramps or other menstrual difficulties.

Three patients (M. B., M. N. and P. R.) have not yet menstruated. M. B. began cortisone therapy at the age of 8½ years and has received it for seventeen months. Normal adolescent sexual development has occurred, but menstruation has not begun. M. N., who is 10½ years old, has received treatment for only five months and the 17-ketosteroids have been below 8 mg. per day for only four months. The failure of P. R. to menstruate is of interest. This patient is now 17 years old and has been treated for twelve months. During the first five months of treatment, while she was receiving 50 mg. of oral cortisone daily, the 17-ketosteroids were frequently above the level of 8 mg. per day and her acne and hirsutism did not disappear. For the past seven months, however, adrenal suppression has been maintained more completely with the administration of 75 mg. of oral cortisone daily. It was difficult to determine how much of this patient's breast enlargement was due to glandular tissue and how much

to fat. Likewise, her vaginal smear showed perhaps less estrogenic effect than has occurred in most of the other patients. Since this patient previously had received intensive treatment with both stilbestrol and anhydrohydroxyprogesterone, we wonder whether her pituitary-ovarian mechanism may have been permanently damaged.

*Basal temperature curves.* Only 2 of the patients, J. S. and M. C. S., kept records of their basal temperatures during menstrual cycles. The curves (Fig. 1) showed fluctuations, with a tendency to fall following

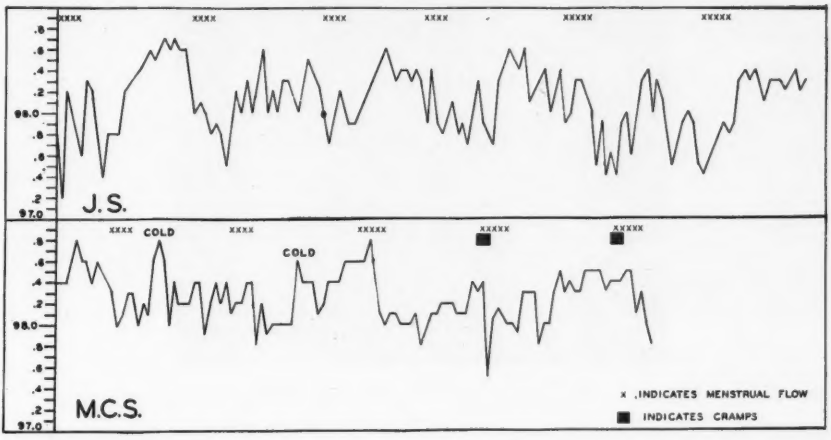


FIG. 1. Basal temperature curves of 2 female pseudohermaphrodites who developed feminine characteristics and had menstrual periods after treatment with cortisone.

menstruation and to rise to a peak at some time between the middle of the cycle and the menstrual period. Several slight intercurrent infections interfered with the regularity of the curves. These findings suggest that ovulation and progesterone secretion occurred, and the premenstrual cramps in the case of M. C. S. makes this seem probable.

*Hair growth and acne.* In the cases of J. S., and M. C. S. the rate of growth of sexual hair was measured by weighing the axillary hair shaved at regular intervals. This indicated that the growth decreased to less than half the pretreatment rate. We do not have great confidence in this as a quantitative method because of various technical difficulties. Nevertheless, observation left no doubt that most patients showed a definite decrease of hirsutism. This was most striking in the cases of J. S. and M. C. S., who had had to resort to electrolysis about once a week for the removal of facial hair. Before treatment M. C. S. had a heavy tuft of coarse black hair beneath the lower lip and coarse dark hair on the sides of her face and beneath the chin. With treatment the hair did not fall out but grew

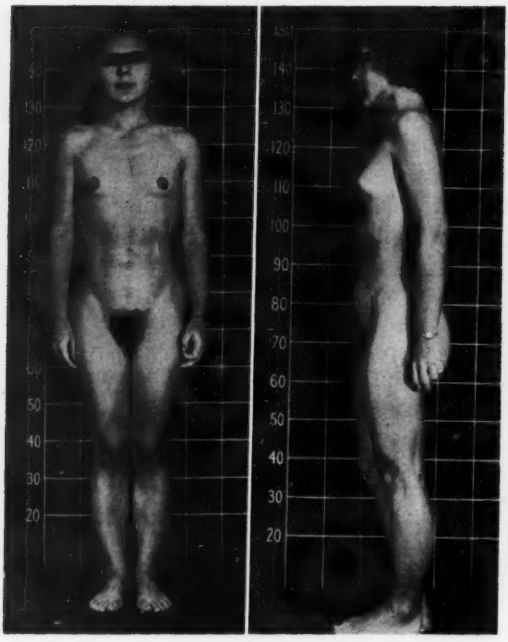


FIG. 2. *Patient J. S.* Age: 18½ years.

*After treatment with cortisone for four months*

Before treatment 17-ketosteroids were 53 mg./24 hrs.

After treatment 17-ketosteroids were 5 mg./24 hrs.

Breasts developed, vaginal smear became estrinized, regular menstruation occurred, pigmentation faded and hypertension disappeared.

more slowly. There was a striking change in its character, so that it became finer and of almost blond color. She no longer took electrolysis treatments. There was a similar change in the character of the hair on her forearms, legs and thighs. Changes in the pubic and abdominal hair were not so noticeable. Between the seventieth and one hundred and twentieth days of treatment, when the dose of cortisone given orally was reduced to 25 mg. a day and the urinary 17-ketosteroids rose to levels between 10 and 18 mg. per day, the hair reverted somewhat to its former coarse, dark character. With a change of treatment to 25 mg. of intramuscular cortisone daily, which caused suppression of the urinary 17-ketosteroids to less than 6 mg. per day, the hair again became finer and lighter. In a number of the patients the scalp hair became finer, more wavy and less oily. Acne and oiliness of the skin disappeared entirely in some of the patients and decreased markedly in others. Like the hair growth, the improvement

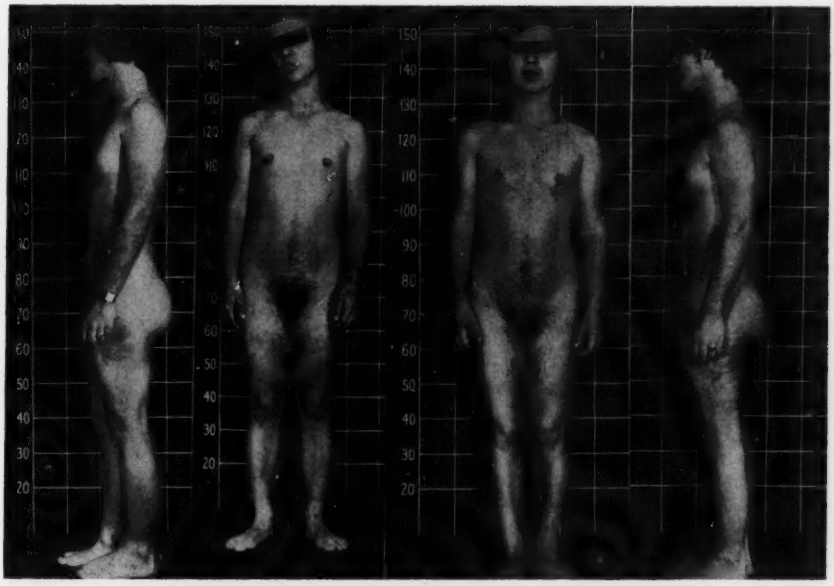

FIG. 3. *Patient M. C. S.* Age: 16¼ years.

*Before treatment* — 17-Ketosteroids: 62 mg./24 hrs.

*After treatment with cortisone for two months* — 17-Ketosteroids: 6 mg./24 hrs.

Patient menstruated regularly, with basal temperature fluctuations and cramps. Breasts developed and vaginal smear became estrinized. Subsequently hirsutism decreased progressively. Hypertension disappeared and Addisonian pigmentation faded.

Photograph illustrates rapidity of the change. Patient's appearance has improved greatly since this time.

seemed to vary somewhat with the degree of suppression of the 17-ketosteroids.

*The clitoris.* All of the female pseudohermaphrodites in the older group had had the clitoris either amputated or completely extirpated before treatment with cortisone. In the case of M. N. the stump was painful before treatment, apparently because of a state of constant erection. The pain disappeared promptly with treatment and the stump no longer was palpable.

*Voice.* The patients all had rather deep "throaty" voices, but none was bass. No change in pitch was noted under treatment.

*Somatic growth and development.* The older female pseudohermaphrodites were not satisfactory subjects for observing the effects of cortisone on skeletal growth and epiphysial development. As shown in Table 1, their epiphyses were completely or almost entirely fused with the long bones. Accordingly, very little or no growth occurred under treatment.

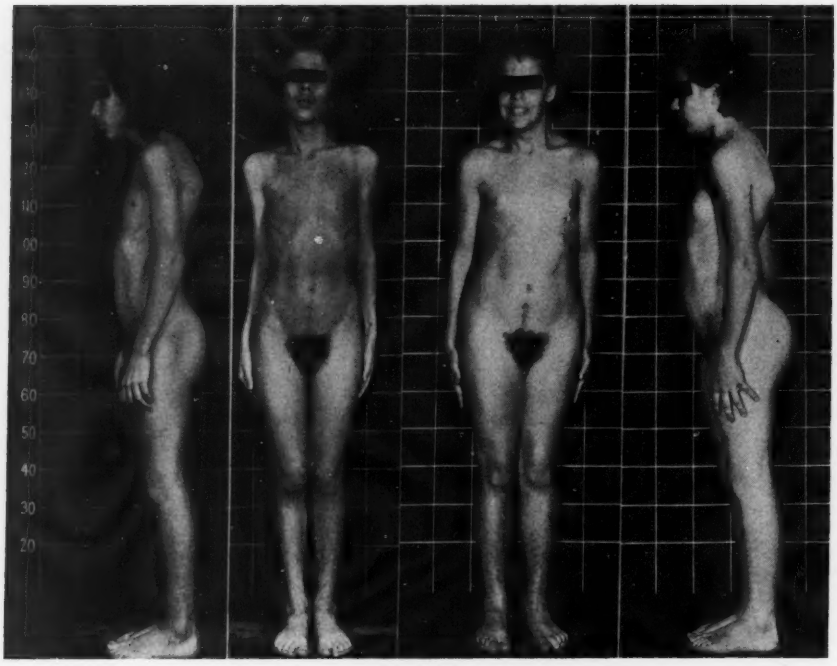

FIG. 4. *Patient W. D.*

| *Before treatment* | *After treatment with cortisone for eight months* |
|---|---|
| Age: 9 years, 2 months. | Age: 9 years, 10 months. |
| 17-Ketosteroids: 30 mg./24 hrs. | 17-Ketosteroids: 4 mg./24 hrs. |

Breasts developed and vaginal smear became estrinized. Four menstrual periods occurred.

Note changes of body contours due to deposition of fat over buttocks and hips. Gluteal striae developed.

(Poor posture is due to paralysis of lumbodorsal muscles resulting from poliomyelitis.)

*Body contours and the development of gluteal striae.* After treatment with cortisone was begun, the feminine contours of the body developed strikingly in some of the cases (Figs. 2, 3 and 4). Not only did the bust develop, but the buttocks and hips became more prominent, causing the waist to be more clearly defined. During this period of change, 2 patients (M. B. and W. D.) showed conspicuous reddish striae in parallel longitudinal lines over the buttocks and in the trochanteric regions. The striae resembled those seen in Cushing's syndrome but occurred nowhere else on the body and were not accompanied by plethora, ecchymoses, generalized obesity, moon-face, hypertension or changes in carbohydrate metabolism.

In the case of M. B. the striae occurred while she was receiving cortisone by intramuscular injection in the buttocks. They persisted for three or four months and then disappeared spontaneously, in spite of the fact that intramuscular injections were continued. At first it was thought that these might represent a local effect of cortisone on the elastic tissue of the skin. This explanation, however, became untenable when W. D., who was receiving cortisone by mouth, showed even more prominent striae. Careful examination has convinced us that they were caused by stretching of the skin due to the rapid development of the depots of fat over the buttocks and hips, which are characteristic of the adult female contours. Apparently in the patients treated with cortisone some of the changes of adolescence progress even more rapidly than they do normally.

*Weight and appetite.* Most of the patients had very large appetites before treatment, although they were of slender build. This was particularly true in the younger patients who previously had grown rapidly. In a number of instances the parents noted a decided decrease of the appetite after treatment with cortisone was begun. Three of the patients had little change of weight under treatment. P. R., who was fairly obese before treatment, gained 2.5 Kg. during the first seven months of therapy. With restriction of diet, her weight became stabilized. W. D., who had previously been very slender, gained 6.6 Kg. under treatment but did not seem obese. M. C. S. gained 4.5 Kg. between the fortieth and sixtieth days of treatment, while she was receiving 50 mg. of cortisone daily by mouth. At the same time the blood pressure, which had fallen to normal during cortisone treatment, rose to its previous hypertensive levels. When the dose of cortisone was decreased and the sodium intake restricted, the weight decreased to the previous level and the blood pressure fell.

*Hypertension.* Two of the patients in Group A (J. S. and M. C. S.) and 1 boy in Group C (W. J.) had considerable hypertension. Under cortisone therapy the hypertension disappeared in all 3 cases. This observation will be reported in detail in a subsequent communication.

*Pigmentation.* Both sisters, J. S. and M. C. S., had marked generalized brownish pigmentation, accentuated in the lines of the palms, over the knuckles and in other folds of the skin, similar to that seen in Addison's disease. There was also brownish pigmentation of the gingival mucosa. Rapid depigmentation occurred in both cases.

*Metabolic or toxic effects of cortisone.* In the 6 older female pseudohermaphrodites under discussion, no abnormalities of carbohydrate metabolism were noted, as determined by glucose tolerance tests. No changes in serum electrolytes were found. The only case in which it was suspected that there might be effects of excessive cortisone was that of M. C. S., who gained 4.5 Kg. of weight rapidly, with development of a

full face and recurrence of hypertension, while receiving 50 mg. of cortisone daily by mouth. The fact that weight was lost and the blood pressure fell when her sodium intake was restricted later suggested that the symptoms might have been due to salt retention, in spite of the fact that the serum electrolyte values were normal. Subsequently the patient has been maintained on 25 mg. of intramuscular cortisone per day without restriction of salt and has had no recurrence of hypertension or edema.

The peculiar occurrence of striae localized on the buttocks and hips which has been discussed previously cannot be considered a toxic manifestation of cortisone therapy, since there were no other evidences of Cushing's syndrome. Probably the striae are caused by skin distention due to rapid deposition of fat in these regions, similar to that which occurs normally but more slowly in females during adolescence.

### GROUP B—INFANTS WITH FEMALE PSEUDOHERMAPHRODISM AND ELECTROLYTE-REGULATING DEFECTS

The data concerning the 3 patients in this group are listed in Table 2. The symptomatology and therapeutic problems in these cases differed considerably from those in the older female pseudohermaphrodites who had no evidence of electrolyte disturbances. These infants were all admitted to the hospital in states of dehydration with evidences of marked sodium loss. The immediate problem was to correct the electrolyte defect. The effects of cortisone, high sodium intake, desoxycorticosterone and compound B (corticosterone) on the electrolyte regulation were studied extensively and will be reported in another paper. These patients presented an opportunity to study the effects of cortisone on somatic growth and development, an opportunity which was not afforded by the older female pseudohermaphrodites, whose growth had entirely or nearly ceased. The infants, however, were less favorable subjects for observing the effects of cortisone on sexual development during the relatively short periods of study.

*Sexual changes.* Before treatment there were minor variations in the configuration of the external genitalia in the 3 patients. Each of them had a markedly enlarged clitoris resembling a hypospadic penis. In each case there was a small urogenital sinus with a urethral-like meatus, and its communication with a vagina was demonstrated by urethroscopic examination. The degree to which the labia majora were fused posteriorly and the size of the vulvar groove varied. In 2 cases (F. F. and L. U.) there was considerable fusion, with the formation of a median raphe. In F. F. the resemblance of the fused labia to a scrotum, and the location of the urogenital orifice at the base of the phallus, led one of us (L. W.) to suspect that the patient was a male pseudohermaphrodite when she was examined

TABLE 2. GROUP B—YOUNG INFANTS WITH FEMALE PSEUDOHERMAPHRODISM AND SALT LOSS

| Before treatment | | | | | | | Treatment with cortisone | | | | | Results of treatment | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Name | Age | Height age | Bone age | Wt. (Kg.) | 17-KS, mg./day | Associated symptoms | Date begun | Route | Average daily dose (mg.) | How given | Duration (mos.) | 17-KS, mg./day | Sex development | Growth, (in.) | Weight change (Kg.) |
| L.U. | 11 wks. | 8 wks. | 9 wks. | 3.6 | 3.5 | Salt loss | Birth | | | | 15 | 0.4 | No change | 8.7 | +4.4 |
| | | | | | | | 8-14-50 | i.m. | 25.0 | 25.0 q d | | 0.3 | | | |
| | | | | | | | 12- 5-50 | i.m. | 5.0 | 5.0 q d | | 0.6 | | | |
| | | | | | | | 2-14-51 | 0 | 5.0 | 2.5×2 | | 3.3 | | | |
| | | | | | | | 3-29-51 | — | 0.0 | — | | 1.2 | | | |
| | | | | | | | 4-30-51 | 0 | 5.0 | 2.5×2 | | 2.9 | | | |
| | | | | | | | 5-21-51 | — | 0.0 | — | | 1.3 | | | |
| | | | | | | | 6-13-51 | i.m. | 12.5 comp. B | q d | | 1.0 | | | |
| | | | | | | | 8-24-51 | i.m. | 25.0 comp. B | q d | | 1.8 | | | |
| | | | | | | | 9- 5-51 | i.m. | 5.0 comp. B | q d | | 6.0 | | | |
| | | | | | | | 9-21-51 | — | 0.0 | — | | 1.7 | | | |
| | | | | | | | 9-28-51 | 0 | 12.0 cortisone | 4.0×3 | | | | | |
| F.F. | 19 wks. | 19 wks. | 13 wks. | 5.8 | 4.8 | Salt loss | Birth | | | | 10 | 1.4 | No change | 6.5 | +3.2 |
| | | | | | | | 8-23-50 | | | | | 1.1 | | | |
| | | | | | | | 1- 3-51 | 0 | 25.0 | 12.5×2 | | | | | |
| | | | | | | | 1-29-51 | 0 | 12.0 | 6.0×2 | | | | | |
| S.K. | 11 wks. | 5 wks. | | 4.2 | 3.2 | Salt loss | Birth | | | | 9 | 1.7 | No change | 7.5 | +5.5 |
| | | | | | | | 11-20-50 | | | | | 0.5 | | | |
| | | | | | | | 2- 9-51 | 0 | 12.0 | 6.0×2 | | 0.3 | | | |
| | | | | | | | 2-28-51 | i.m. | 12.5 | 12.5 q d | | 1.3 | | | |
| | | | | | | | 3-28-51 | i.m. | 8.3 | 25.0 q 3 d | | | | | |
| | | | | | | | 6-12-51 | i.m. | 6.25 | 25.0 q 4 d | | | | | |

TABLE 3. GROUP C—MALES WITH MACROGENITOSOMIA PRAECOX

| Before treatment | | | | | | | Treatment with cortisone | | | | | Results of treatment | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Name | Age (yrs.) | Height age | Bone age | Wt. (Kg.) | 17-KS, mg./day | Associated symptoms | Date begun | Route | Average daily dose (mg.) | How given | Duration (mos.) | 17-KS, mg./day | Sex development | Growth, (in.) | Weight change (Kg.) | Other effects |
| W.J. | 4½ | 7½ | 11 | 25 | 8.6→2.8 | Hypertension; cardiac failure | 12-22-50 | i.m. | 12.5 | 12.5 q d | 10 | 1.3 | No definite change in secondary sex development. Testis grew slightly | 1.0 | +6 | Decreased B.P. Decreased heart size |
| | | | | | | | 6-16-51 | 0 | 25.0 | 12.5×2 | | 1.2 | | | | |
| T.T. | 6 10/12 | 9½ | 13½ | 27 | 26 | | 5- 8-51 | i.m. | 100.0 | 100.0 q d | 6 | 3.8 | No definite change in secondary sex development. Testes increased from 1.8 ×1.2 cm. to 3.8×2.7 cm. | 0.8 | +4 | |
| | | | | | | | 5-16-51 | i.m. | 25.0 | 50.0 q 2d | | 2.9 | | | | |
| | | | | | | | 6-11-51 | i.m. | 25.0 | 75.0 q 3d | | 3.5 | | | | |

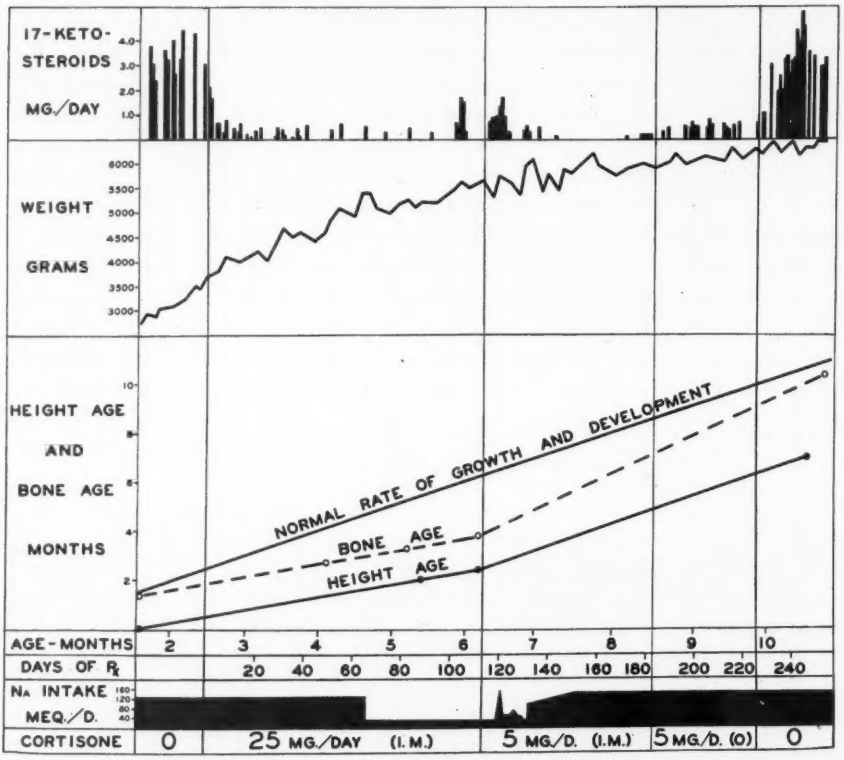


FIG. 5. Chart shows retardation of rates of growth and osseous development of patient L. U. while she was receiving 25 mg. of intramuscular cortisone daily and the increase of rates when the dose was decreased to 5 mg. daily. The dose of 5 mg. daily was sufficient to maintain suppression of the ketosteroid excretion without inhibiting growth and development.

during the first week of life. The diagnosis of congenital adrenal hyperplasia was established by the finding of increased urinary 17-ketosteroids, varying from 3 to 5 mg. per day. Before treatment, only 1 patient (S. K.) had a small amount of blond hair over the pubis and this disappeared under treatment.

During treatment with cortisone, which now has been continued for from ten to fifteen months, no change in sexual development was noted. There was no growth of the phallus but no definite decrease of its size was noted. Erections, which were common before treatment were absent or rare. There was no growth of sexual hair.

*Somatic growth and development.* The effect of cortisone on the growth and osseous development of L. U. is shown in Figure 5. When the infant

came under observation at the age of 7 weeks, her length was 3.2 inches below the average and was that of a newborn infant. The skeletal development also was that of a newborn except for the presence of beginning ossification in the proximal epiphysis of the humerus. The retardation of growth was probably due to the fact that the defective electrolyte regulation with dehydration, frequent vomiting and poor appetite had resulted in a state of malnutrition. When the patient was treated with 25 mg. of intramuscular cortisone daily between the eleventh and twenty-eighth weeks of life, the rate of linear growth continued to lag below the normal rate, so that at 28 weeks her length was only that of an average 9-week-old infant. Roentgenograms of the bones made at the end of this period showed that no new centers had appeared and there was little evidence of growth of the round bones or at the ends of the long bones. During this time, however, she became definitely obese, in spite of the fact that her diet contained only 92 calories per kilogram of body weight. Her fatty cheeks protruded laterally beyond her ears and overhung the mandibles, causing a round "moon face" similar to that seen in patients with Cushing's syndrome. There was no plethora of the skin and no striae appeared. She seemed in good health and was bright, alert and playful, but was perhaps less active and energetic than most infants of her age. There was marked hypotonicity and weakness of the muscles. The systolic blood pressure varied from 110 to 120, and the diastolic from 70 to 85, values which are somewhat elevated for an infant of this age.

When the patient was 28 weeks old the dose of cortisone was reduced to 5 mg. per day, and this proved sufficient to maintain the suppression of urinary 17-ketosteroids below 0.6 mg. per day. With this reduction of dosage, the patient's growth increased at the normal rate with an increment of 4 inches during the next five months, so that at the age of 11 months she had attained the height of an average infant of 7 months. Roentgenograms showed definite growth in the size of the epiphysial centers of ossification, and new centers appeared. There was also obvious new growth at the ends of the long bones. At the age of 11 months her bone age was about no mal for an infant of 1 year. With the smaller dose of cortisone the patient's obesity decreased and her face no longer was abnormally rounded. She became more active and vigorous. The fluctuations of weight shown on the chart were accounted for by electrolyte and fluid changes, to be discussed in a subsequent paper.

In the 2 other infants, cortisone caused no obvious retardation of growth and development. S. K., who received 25 mg. of intramuscular cortisone every fourth day (equivalent to 6.25 mg. daily), at the age of 12 months had average measurements for her age, with a height of 29 inches and weight of 21.4 pounds. Six teeth were present and the anterior fontanelle

was nearly closed. The osseous development was somewhat advanced, with calcification of the great tuberosity of the humerus, the distal epiphysis of the fibula and all three cuneiforms of the foot. F. F., who received 12 mg. of oral cortisone daily, at the age of 14 months had a height of 29 inches and weight of 16 pounds. Her osseous development was not significantly abnormal; the distal epiphysis of the tibia was calcified but that of the radius was not.

The effects of cortisone on the growth and development of these infants is of importance. It is apparent that in the case of L. U. the dose of 25 mg. of intramuscular cortisone inhibited normal growth and development and caused obesity and symptoms suggestive of Cushing's syndrome. On the other hand, 5 mg. of cortisone daily proved sufficient to prevent the excessive excretion of 17-ketosteroids without inhibiting normal growth. More time must elaspe before one can assess adequately the effects of prolonged cortisone therapy on the somatic growth and development of these patients. It is well known that in the adrenogenital syndrome, growth, osseous development and muscular development are exceedingly rapid due to the protein-anabolic effects of excessive androgen, whereas in Cushing's syndrome these are all retarded because of the predominance of glycogenetic over androgenic hormones. Silverman, Day and Blodi (3) have reported that the administration of large amounts of ACTH to premature infants with retrolental fibroplasia caused inhibition of growth. Since the objective of cortisone therapy in young children with congenital adrenal hyperplasia should be not only to prevent progressive virilization but also to restore normal somatic growth and development, it is important to follow the rates of growth and epiphysial development together with the urinary 17-ketosteroids, as guides to treatment. Inhibition of growth may indicate that an excessive amount of cortisone is being given, whereas continued excessive growth would suggest inadequate dosage.

### GROUP C—BOYS WITH MACROGENITOSOMIA PRAECOX

Only 2 male patients have been treated for extensive periods (Table 3). Three other males treated for only two to six weeks are not included in this report.

T. T. was 6 years and 10 months old at the time cortisone was begun on May 8, 1951. His height was that of a 9½-year-old boy and his bone age was about 13 to 14 years. The penis was large and well developed but the testes were relatively small, measuring 1.8×1.2 cm. Pubic hair, which had appeared at the age of 3½ years, was fairly abundant. A testicular biopsy before treatment showed absence of interstitial cells of Leydig. The tubules were of moderate size and showed small lumina. Spermatogonia and Sertoli cells were present, but no mitoses or spermatogenesis

were seen. For six months, while he received 75 mg. of intramuscular cortisone every third day, the output of urinary 17-ketosteroids decreased from 26 mg. per day before treatment to levels of 2.5 to 3.5 mg. per day. Between April 17, 1951, and June 11, 1951, his growth was 1.5 inches; between June 11 and September 10 it was 0.3 inch. The osseous development did not change appreciably after treatment was begun. On September 10, four months after beginning cortisone, it was noted that the size of the penis and prostate, the amount of sexual hair and other secondary sexual development had not changed. On the other hand, the testes had grown remarkably and measured $3.8 \times 2.7$ cm. It will be of interest to repeat the testicular biopsy.

The findings described suggest that cortisone not only suppressed the excessive secretion of adrenal androgen responsible for the rapid growth and virilization, but at the same time led to adolescent development and growth of the patient's testes. The mechanism whereby cortisone may cause adolescent development of the gonads in both males and females will be discussed in a later section.

W. J. had congenital adrenal hyperplasia with macrogenitosomia praecox complicated by severe hypertension, which led to cardiac enlargement and decompensation. At the age of 22 months a large left adrenal was removed without any improvement. In February 1950 he was treated for ten days with intramuscular cortisone in doses of 50 mg., which we believe is an excessive amount. This caused excitement and increased hypertension. Following this he had repeated infections and a downhill course with progressive cardiac failure in spite of the use of digitalis. Between March and December 1950, while he was generally in a state of poor health with cardiac decompensation, the urinary 17-ketosteroids which had previously varied between 6 and 17 mg. per day decreased to values of 1 to 3.5 mg. per day. There was no apparent progress of virilization, the genitalia and pubic hair remaining about the same. On the other hand, he continued to grow rather rapidly, increasing from 46 inches in January 1950 to 48 inches in September 1950. At the latter time when he was 5 years and 4 months old, his height-age was $7\frac{1}{2}$ years. His bone-age advanced from $9\frac{1}{2}$ years in August 1949 to 11 years in December 1950. In December 1950, when death seemed imminent, intramuscular cortisone in doses of 12.5 mg. a day was tried as a last resort. This controlled the hypertension and caused a surprising improvement in the cardiac condition and general health. Treatment has been continued. The urinary 17-ketosteroids have been maintained at levels between 0.5 and 2.4 mg. per day, with an average of 1.3 mg. per day. It is difficult as yet to assess the effect of cortisone on either his sexual or somatic development. Between September 1950 and September 1951 his height increased 1.3 inches, which is a less

rapid rate of growth than before treatment. Between December 1950 and November 1951, while receiving cortisone, the osseous development increased from 11 years to about 11½ years.

### HYPOTHESIS CONCERNING THE ACTION OF CORTISONE IN BRINGING ABOUT FEMINIZATION

It is unlikely that the development of feminine sex characteristics, with the establishment of menstrual cycles, following cortisone therapy is due to a direct action of this hormone on the sex organs or the ovaries. We have previously set forth the hypothesis illustrated in Figure 6.

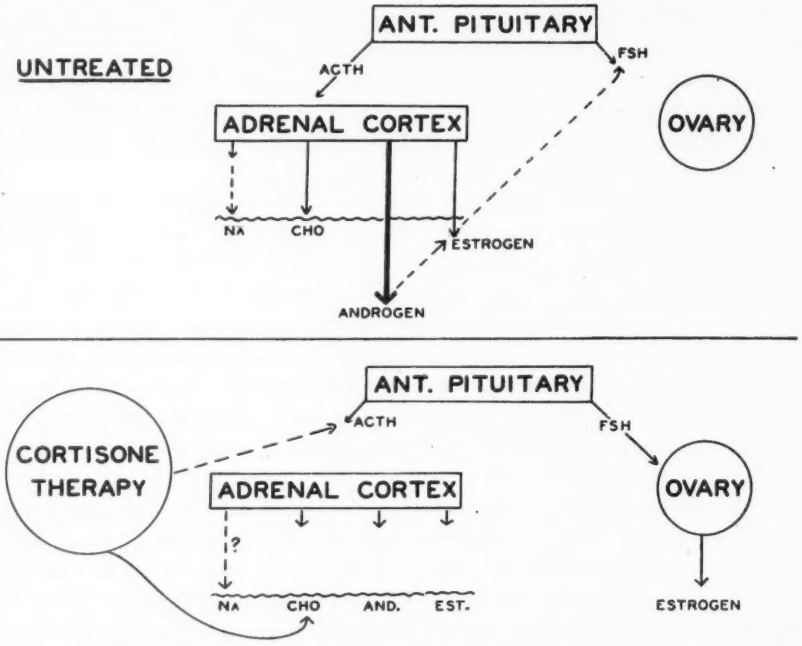


FIG. 6. Diagram illustrates hypothesis of the action of cortisone in bringing about normal feminization of pseudohermaphrodites.

Before treatment the secretion of both androgen and estrogen exceeds the normal (wavy line). The effects of estrogen on the sex organs are prevented by the excessive output of androgen. The excessive amounts of estrogen and androgen inhibit the secretion of pituitary gonadotropins (FSH) so that the ovaries are not stimulated to secrete estrogen.

Cortisone therapy inhibits the effects of ACTH on the adrenal, diminishing its secretions. It also supplies the normal needs of the body for carbohydrate-regulating hormone (CHO). The decrease of excessive androgen and estrogen of the adrenal releases the normal pituitary-ovarian mechanism.

In congenital hyperplasia the adrenal glands probably secrete abnormally large amounts of estrogens in addition to androgen, since both chemical and biologic assay methods show a high output of these hormones in the urine (1). Whether the estrogens secreted by the adrenal are identical with those of the ovary is not known. The estrogens, however, would appear unable to exert an effect on the sex organs because of the antagonism of much greater amounts of adrenal androgen. The presence in the circulation of large amounts of estrogen together with androgen would be expected to inhibit the secretion of gonadotropic hormones by the pituitary. In the absence of gonadotropin, the ovaries (or testes) would remain immature and secrete no female hormone.

The administration of cortisone probably inhibits the secretion of ACTH by the pituitary (4). In addition it may be capable of preventing the action of administered ACTH upon the adrenal, as suggested by an experiment on patient L. U., previously reported (2). With the inhibition of ACTH, the hyperactive adrenal is put at rest, although possibly the secretion of electrolyte-regulating hormones continues. The metabolic requirements of the body for adrenal glycogenetic hormones are fulfilled by cortisone, when it is given in proper doses. If an excessive amount is given, however, symptoms of Cushing's syndrome may result or the normal level of protein anabolism may be suppressed, with inhibition of somatic growth and development. With suppression of adrenal activity the secretion of adrenal androgen and estrogen decreases markedly. This permits the secretion and release of pituitary gonadotropins. These cause the maturation of the ovary and the secretion of estrogen. The ovarian estrogen is no longer antagonized by excessive adrenal androgen, and normal secondary sex development results. The normal pituitary-ovarian mechanism leads eventually to the establishment of menstrual cycles with ovulation, corpus luteum formation and progesterone secretion. A similar mechanism in the male may be expected to lead to maturation of the testes with spermatogenesis. In this case, adolescent amounts of testicular androgen may be secreted, while the excessive amounts of adrenal androgen are suppressed. It is probable that unless boys with congenital adrenal hyperplasia are treated with cortisone at an early age, they will continue to show progressive development of male sexual characteristics, such as occurs in normal adolescence.

The fact that, with cortisone, female sexual development occurred rapidly in 3 pseudohermaphrodites between $8\frac{1}{2}$ and $10\frac{1}{2}$ years of age and that testicular development began in a boy of 7 years, may be due to the fact that these patients had attained a somatic and osseous development of from 13 to 15 years, due to the accelerating effects of excessive androgen. Apparently the pituitary-gonadal mechanism had reached a state of

maturity and was capable of functioning promptly as soon as the inhibitive effects of adrenal hormones were suppressed.

In an attempt to verify these speculations, extensive studies of the gonadotropins and estrogens are under way. In several cases we have been able to demonstrate after cortisone treatment the presence of urinary gonadotropins which were absent before treatment.

## SUMMARY

1. Six female pseudohermaphrodites between the ages of $8\frac{1}{2}$ and $18\frac{1}{2}$ years have been treated continuously with cortisone for periods of from five to seventeen months. In all, the breasts promptly developed and the vaginal smears showed estrinization. Three of these patients have menstruated regularly. There has been an unquestionable decrease of hirsutism in all patients—in 2, to a spectacular degree.

2. To obtain the most satisfactory results, it is essential to give the minimum amount of cortisone necessary to maintain the excretion of 17-ketosteroids below 8 mg. per day in the older patients, as we have seen a recurrence of androgenic manifestations when larger amounts of ketosteroids were excreted. The methods of determining the proper maintenance dose are discussed elsewhere (2).

3. The older patients were unsatisfactory for investigating the effects of cortisone on somatic growth and development, because their epiphyses were fused; but 3 young infants with female pseudohermaphrodism were excellent subjects for this study. In 1 infant, 25 mg. of intramuscular cortisone daily inhibited growth and osseous development, but 5 mg. proved sufficient to suppress excessive androgen secretion without inhibiting normal growth. During childhood it is advisable to follow both the urinary 17-ketosteroids and the rates of growth and osseous development as guides to treatment. The minimal effective dose of cortisone and the level at which the urinary 17-ketosteroids should be maintained must be determined individually in each case.

4. In 1 of 2 boys treated with cortisone there was enlargement of the testes, suggesting that normal maturation was occurring.

5. Cortisone caused a marked decrease of hypertension in 3 cases.

6. In 2 cases pigmentation of an Addisonian type disappeared under cortisone therapy.

7. Evidences of excessive metabolic effects of cortisone were observed in 1 young infant who received daily intramuscular injections of 25 mg. of cortisone. These effects disappeared when the dose was reduced. One girl of 17 years had a temporary increase of weight and recurrence of hypertension while receiving 50 mg. of cortisone daily by mouth.

8. Extensive striae developed on the buttocks and hips of 2 girls who

were showing rapid female development. This was not considered to be a toxic effect of cortisone, but was probably due to the rapid deposition of fat in these regions, similar to that which occurs in normal females during adolescence.

9. The effects of cortisone and other steroids on the electrolytes were studied in 3 infant female pseudohermaphrodites of the "salt-losing type" and will be reported elsewhere.

10. An hypothesis is offered to explain the action of cortisone in bringing about normal sex development in patients with this condition.

*Acknowledgments*

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# ADRENAL CORTICAL FUNCTION IN HYPERTENSION

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## INTRODUCTION

THERE is an impressive body of suggestive evidence for the importance of the adrenal cortex in various types of hypertension. The subject has been comprehensively reviewed by Sapeika (1) and Perera (2). In the present communication, some of the relevant clinical and experimental observations will be briefly recapitulated in order to place this investigation in its proper perspective.

Pressor effects of desoxycorticosterone esters have been demonstrated in various animals (3, 4), and morphologic changes were first described by Selye *et al.* (5). These included generalized vascular lesions similar to those seen in human nephrosclerosis and in malignant hypertension. Subsequently, studies by Knowlton *et al.* (6), employing much lower dosages in rats, revealed no significant vascular lesions; but in nephritic rats (the nephritis being induced by cytotoxic serum) striking hypertension was readily induced. The development of these lesions can be inhibited by simultaneous administration of glucocorticoids or ACTH (7, 8).

Loeb *et al.* (9) first reported hypertension in 2 patients suffering from Addison's disease, who were treated with desoxycorticosterone esters. This pressor effect has also been demonstrated in uncomplicated hypertensive vascular disease, but not in normotensive subjects (10). The elevation was masked by salt withdrawal and apparently blocked by simultaneous administration of adrenal cortical extract (11). Cortisone in a dose of 200 mg. per day was found to cause a small but definite depression of the blood pressure in a patient with hypertensive vascular disease, which persisted for several weeks after cortisone had been discontinued (12).

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† The Section of Mathematical Statistics, Commonwealth Scientific and Industrial Research Organization, University of Adelaide, is responsible for the statistical analysis.

Such an accumulation of suggestive evidence for the importance of the adrenal cortex in hypertension indicates the need for information as to the state of adrenal cortical function in this disease. This investigation, on which a preliminary communication has already appeared (13), was undertaken as a contribution to the problem.

The studies reported on adrenal cortical function in hypertension fall into two main categories, according to the methods employed for evaluation, *viz.*, indirect or direct methods.

A number of investigations using indirect methods have been reported (14–18). Direct methods include various techniques for estimation of hormone levels in body fluids, usually urine. Two main types of estimation are available; chemical methods or biologic assay.

Chemical methods are much easier and quicker, but have the disadvantage that they are not specific for the various steroids having different physiologic effects, *i.e.*, they cannot distinguish between salt-active and glucocorticoid fractions. The reports that are available include those of Tobian and Joseph (19) and Corcoran *et al.* (20); both groups used the formaldehydogenic method.

Biologic assay is more tedious, and unfortunately methods sensitive enough for estimations in body fluids have been difficult to develop, especially for salt-active corticoids. Several procedures are available for estimation of glucocorticoids, including that of Venning *et al.* (21), which has been used in this investigation. Results obtained with this method in a series of cases of hypertension have been reported by F. L. Selye (15).

The estimation of 17-ketosteroid excretion was undertaken as a subsidiary investigation. A small decrease in the excretion has been reported (22).

## CLINICAL ASPECTS

The series of cases of hypertension, on some of which glucocorticoid and 17-ketosteroid estimations were carried out, comprised 203 patients seen in the Royal Adelaide Hospital over a period of nearly two years. They were all personally examined by one of us and full clinical details have been given elsewhere (23).

Patients suffering from severe congestive cardiac failure or renal failure were not considered suitable for these estimations. Gross cerebral damage naturally precluded investigation, as accurate 24-hour or 48-hour specimens of urine could not be obtained.

The patients on whom these estimations were made were relatively early cases of severe hypertension, with resting blood pressure equal to or greater than 180 mm. of Hg systolic and 120 mm. diastolic. Headache or such complications as cerebrovascular accident or early congestive cardiac

failure had occurred in all cases. A number of the patients were ambulatory, and some patients had just undergone various types of sympathectomy.

In the whole series of 203 cases, there were only 22 cases of renal hypertension and 1 case of pituitary basophilism, leaving 180 regarded as "primary" or "essential" hypertension. Certain groupings have been made of these cases, the criteria for which have been fully discussed elsewhere (23). An attempt has been made to see whether there was any variation of adrenal cortical function in these different groups, as we have no reason to believe that "essential" hypertension is a single entity.

As controls for the glucocorticoid assay normotensive patients have been used, with a few healthy normotensive ambulatory men also included. Factors likely to cause a disturbance of adrenal cortical function, such as acute infection or recent trauma, were excluded as far as possible, but exposure to some forms of stress, *e.g.*, physical exercise or emotional excitement, could not be excluded in the ambulatory controls or in the hypertensive patients. It is considered that such factors may have been responsible for some of the high values obtained.

The age distribution of the series has been discussed fully elsewhere (23). In the patients on whom these estimations were made the range in age was from 22 to 52 years, with the exception of 2 patients aged 16 and 14 years. The ages of the controls were in the same range.

## METHODS

### 1. *Biologic assay of glucocorticoids in urine*

The assay depends upon the deposition by adrenal cortical hormone of liver glycogen in the fasting adrenalectomized mouse. This principle was used first by Reinecke and Kendall (24) with rats, but by using mice and a standard dose of glucose the technique has been made much more sensitive and hence applicable to the assay of adrenal cortical activity in urine.

The procedure used followed that of Venning *et al.* (21), with minor modifications.

Male white mice (20–26 Gm.) from our own colony were used. This colony was not an inbred one, but was considered sufficiently homogeneous for the purpose. The mice were adrenalectomized by way of the lumbar route using ether anesthesia, which was found much more convenient than amytal for an operation lasting three or four minutes. The mortality was negligible. After operation and until killed, the mice were kept in a constant-temperature room, thermostatically controlled at $80° \pm 4°$ F. The diet contained 20 per cent protein and the drinking water consisted of 1 per cent salt in tap water.

At 5 p.m. on the third postoperative day the mice were fasted overnight

for at least sixteen hours, but continued to receive saline. The saline was removed at the commencement of injections at 9:30 a.m. on the next day. Then, over a period of six hours the extract was injected subcutaneously in 4 doses of 0.2 ml. each at approximately 1½-hour intervals. At the end of this period the mice were anesthetized with sodium amytal (0.2 ml. of 1.5 per cent, intraperitoneally). The livers were quickly removed, dropped into waiting tubes containing 4 ml. of hot 30 per cent potassium hydroxide, and boiled.

The liver glycogen was precipitated with alcohol overnight in the cold room, using the method of Good, Kramer and Somogyi (25). After hydrolysis the glucose was estimated by the Schaffer-Hartman (26) method. The accuracy of the method over the range of the assay was checked by estimation of standard quantities of glycogen, and good recoveries were obtained.

Urine specimens were collected generally over forty-eight hours and, whenever possible, without preservative. During the summer months it was sometimes necessary to use a small amount of normal HCl as preservative; specimens collected in this manner were never more acid when received at the laboratory than those successfully collected without preservative. Occasional alkaline specimens were discarded and the urine was collected over again. The pH was adjusted to 1, using N/10 HCl as the control, with brom-thymol blue as an indicator. The urine was left standing overnight at room temperature, as this was found to reduce considerably the likelihood of emulsions forming during extraction. If there was any sediment, it was filtered for the same reason.

The specimen was then extracted four times with fresh chloroform by hand-shaking with volumes at least 1/6 the volume of the urine, washed and dried ready for assay according to the standard procedure of Venning *et al.* (21). The dry residue was stored in the cold until required for assay, when it was dissolved in 0.36 ml. of absolute alcohol (kept to a minimum, as it was toxic for the mice) and the standard dose of 60 mg. glucose added; the volume was then made up with water to give each of 6 mice 0.2 ml. four times. The final concentration of alcohol was thus 7.5 per cent.

*Standardization.* First of all, the fasting levels of liver glycogen were checked and were found to be minimal both at the beginning and at the end of the injection period, on batches of from 26 to 30 mice. Levels were below 5 mg./100 Gm. with very few exceptions, which may explain the occasional high individual mouse values in the assay.

The next step was the determination of a dose of glucose which would not affect this basic level of liver glycogen when given by itself. Doses of 40 mg., 60 mg., 80 mg., 100 mg. and 120 mg. were tested. There was some doubt about 80 mg. and 100 mg., so 60 mg. was decided on as a safe dose.

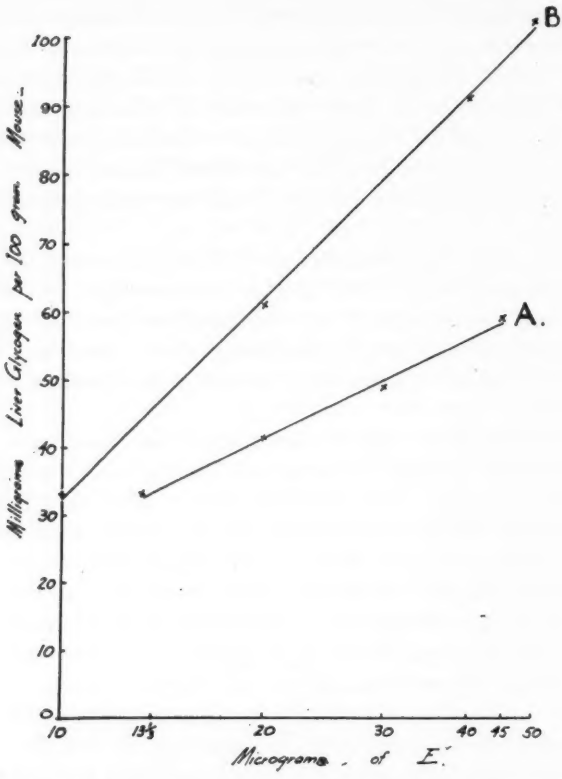


FIG. 1. Logarithmic dose-response regression line for adrenal cortical extract (A) compared with that (B) of Venning *et al.* (21).

The mouse response was first tested against a whole adrenal cortical extract (Eschatin, of Parke, Davis & Co.) which had been standardized commercially against 11-dehydro-17-hydroxycorticosterone (compound E) by the rat glycogen method (27); 1 ml. was found equivalent to 200 micrograms (μg.). A range of dosage over 5 to 80 mg. was tested and the statistical analysis of these data showed that although the mean depositions of glycogen in 6 to 8 mice at different dose levels were significantly different, there was a large within-group variation.

It was considered possible that some of this variation might be due to the use of a glandular extract rather than a crystalline steroid and therefore, when a supply of Cortone Acetate (11-dehydro-17-hydroxycorticosterone acetate) became available,[1] further tests were performed, using this material

[1] Kindly supplied by Dr. James Carlisle of Merck & Co., Rahway, New Jersey, U.S.A.

as a standard. However, a similar range of variation was observed in the analysis of these data. The regression line was not as steep as that of Venning *et al.* (21). This was attributed to slow absorption of the cortisone acetate, so that it was decided to revert to cortical extract for further standardization.

The analysis of subsequent block experiments with Eschatin showed that some of the within-group variation was due to a day-to-day variation in the response of the mice. The analysis showed that with 24 mice a remarkably good regression line was obtained (Fig. 1), but with only 6 mice there was again considerable variation at one dose level.[2]

It will be seen from Figure 1 that increasing doses of adrenal cortical hormone did not give very large increments in liver glycogen, so that the regression line was again not as steep as that of Venning *et al.* (21). Venning, in a personal communication, has suggested that this may have been due to the preservative (0.5 per cent phenol) interfering with the glycogen deposition in the liver as the dosage was increased.

Unfortunately adrenal cortical extract free of preservative (used by Venning as a standard) was not available to us, so that we could not prove this to be so. This possibility, however, is supported by the fact that better agreement is obtained between our figures for the normal range and the figures of Venning and Kazmin (29) both for adults and for children (30), when the regression of Venning *et al.* (21) is used. As will be seen from Figure 1, the difference was not very great at the lower dosage levels, but at the higher dosage levels it was considerable, *e.g.*, a difference of 10 mg./100 Gm. of glycogen between 40 and 50 mg. makes a difference of 11 $\mu$g., as opposed to 3 $\mu$g. with the line of Venning *et al.* (21).

We have, however, used our own regression when expressing the liver glycogen in micrograms in Tables 1 and 2, but this difference has to be borne in mind when considering the microgram-equivalents for glycogen depositions above 40 mg./100 Gm. Because of these differences statistical analysis was carried out on our liver glycogen figures. A mean deposition of less than 20 mg./100 Gm. was not considered significant, and all such assays were repeated if possible. Satisfactory results were then obtained in most cases.

2. *The estimation of 17-ketosteroids in urine*

The method followed was a modification of the standard procedure of Holtorff and Koch (31). No correction factor was applied for nonspecific chromogens, as absolute alcohol was not used in the reagents (32).

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[2] Since this work was completed, similar findings have been reported by Sprechler (28).

## RESULTS

### 1. *Glucocorticoid excretion in urine*

Male and female patients will be considered separately.

(*a*). The results in a series of 14 men with hypertension and a group of 11 men with normal blood pressure are shown in Table 1. Figures are given for the mean glycogen deposition of an extract equivalent to an 8-hour urine per mouse and the equivalent activity of adrenal cortical extract expressed as 11-dehydro-17-hydroxycorticosterone in micrograms per twenty-four hours.

TABLE 1. GLUCOCORTICOID EXCRETION IN MEN WITH HYPERTENSION

| Clinical type | No. of cases | No. of assays | Mouse liver* glycogen, mg./100 Gm. | Gluco-corticoid excretion, μg./24 hrs.** |
|---|---|---|---|---|
| *Normal blood pressure* | *11* | *13* | *34.3±2.6* | *42* |
| *Hypertension:* | | | | |
| "Essential" | 11 | 11 | 43.0±3.9 | 64 |
| Renal | 3 | 3 | 36.5±3.6 | 46 |
| Malignant | 1 | 1 | 45.5 | 72 |
| Post-sympathectomy | 5 | 5 | 49.2±7.3 | 84 |
| *Total* | *14* | *14* | *41.6±3.2* | *60* |

* Mean, with its standard deviation, of 6 mice, each injected with equivalent of 8-hour urine.

** Equivalent activity for mean liver glycogen expressed as 11-dehydro-17-hydroxy-corticosterone.

The results in the normal group are rather lower than would have been anticipated on the basis of the findings of Venning and Kazmin (29), who found the normal range to be from 40 to 85 μg./day with an average of 60 μg./day. The results in the pooled hypertension group, however, are very close to the normal average of Venning and Kazmin (29). Statistical analysis showed that there was no significant difference from the normal group. The results in the renal group are somewhat lower than in the "essential" group but not significantly so.

In those patients in whom assays were made following sympathectomy, no significant difference from the results in patients who had not had the operation could be demonstrated. The mean value is rather higher, but this is due to one very high value—a fact indicated by the much higher standard deviation of this group than the others.

(*b*). The results in a series of 27 female patients with hypertension are

shown in Table 2, together with those of a series of 10 patients with normal blood pressure.

The results in patients with normal blood pressure are slightly higher than those obtained by Venning and Kazmin (29), whose figures were from 25 to 65 μg., with an average of 40 μg./day.

TABLE 2. GLUCOCORTICOID EXCRETION IN WOMEN WITH HYPERTENSION

| Clinical type | No. of cases | No. of assays | Mouse liver* glycogen, mg./100 Gm. | Glucocorticoid excretion, μg./24 hrs.** |
|---|---|---|---|---|
| *Normal blood pressure* | *10* | *10* | *38.3± 4.2* | *51* |
| *Hypertension:* | | | | |
| History of toxemia | 10 | 10 | 33.8± 3.1 | 40 |
| No toxemia | 10 | 10 | 36.2± 3.1 | 46 |
| Never pregnant | 6 | 7 | 49.2± 8.0 | 86 |
| Malignant | 3 | 4 | 47.2±10.5 | 79 |
| Post-sympathectomy | 9 | 9 | 39.3± 4.8 | 53 |
| *Total* | *26* | *27* | *38.7± 2.8* | *52* |
| *Cushing's syndrome* | *1* | *1* | *52.3*† | *400* |

*, ** As for Table 1.

† Mean of 6 mice each injected with equivalent of 2-hour urine.

There was no significant difference between the normal and pooled hypertensive groups. It was considered of interest to break up the hypertensive group into those with a history of toxemia of pregnancy, those without such a history, and those who had never been pregnant. However, statistical analysis failed to disclose any significant differences, the high value for those "never pregnant" being associated with a high standard deviation due to several high figures.

There was no significant difference between those cases which showed the malignant hypertensive syndrome and those which did not. The high values were again correlated with a high standard deviation.

In marked contrast to these essentially normal findings are the results obtained in a case of Cushing's syndrome due to pituitary basophilism, shown in Table 2. The glucocorticoid excretion was 8 times the normal mean—400 μg./day.

As an addendum to these results, we wish to make it clear that in the analysis of these data, with groups of 10 cases, differences would have to be greater than 30 per cent to be statistically significant. This is due to the great variability between mice similarly treated and between patients in

any one group. The latter is to be expected in view of the wide range of activity found in normal patients both in this series and in that of Venning and Kazmin (29). This appears to be a reflection of the dynamic state of adrenal cortical function due to factors which are largely uncontrollable, but which produce considerable variation irrespective of the precision of the technique. It is obvious from the results shown in Tables 1 and 2 that there is no correlation between a disturbance of glucocorticoid excretion and any clinical type of hypertensive disease, apart from that occurring in Cushing's syndrome.

2. *17-Ketosteroid excretion in urine*

The results of this estimation in the various clinical types are shown in Table 3.

TABLE 3. 17-KETOSTEROID EXCRETION IN URINE

| Clinical type | No. of cases | No. of estimations | 17-Ketosteroids,* mg./24 hrs. |
|---|---|---|---|
| *Men: "Essential"* | *8* | *8* | *10.8±1.90* |
| *Women:* | | | |
| History of toxemia | 12 | 12 | 8.0±0.70 |
| No history of toxemia | 15 | 15 | 9.0±0.85 |
| Never pregnant | 10 | 10 | 11.1±1.20 |
| Renal | 3 | 3 | 8.7±2.03 |
| Malignant | 4 | 4 | 7.7±0.85 |
| *Total* | *40* | *40* | *9.2±0.52* |

* Mean with its standard deviation.

Mason and Engstrom (32) concluded from a review of the literature that the majority of the mean values for men lie between 12.5 and 16.7 mg./24 hours (range, 6 to 25 mg.), whereas for women the corresponding figures are 7 and 12 mg./24 hours (range, 3 to 22 mg.).

The figures in Table 3 are obviously within these ranges and are not significantly different from normal. Neither are the values for the various groups significantly different from one another.

## DISCUSSION

The evidence for the role of the adrenal cortex in hypertension would suggest three main possibilities for a disturbance of adrenal cortical function in this condition.

1. *Excessive production of glucocorticoids.* The common occurrence of hypertension in Cushing's syndrome, which is associated with excessive gluco-

corticoid excretion (33, 34), raises the possibility that in some cases of essential hypertension there may be an excessive glucocorticoid output from the adrenal cortex. A classic case of Cushing's syndrome with hypertension in a girl of 14, probably due to pituitary basophilism, is included in this series. Her glucocorticoid excretion was 400 μg./day—about eight times the normal output.

Clinical evidence of endocrine disturbance has been found in approximately one third of the women suffering from hypertension in this series. This has been discussed more fully elsewhere (23), but no correlation with an excessive glucocorticoid excretion could be established in such cases.

F. L. Selye (15) found the glucocorticoid excretion raised (100 to 200 μg./day) in 4 of 10 male hypertensive subjects, and in 2 of 8 females (82 μg. and 126 μg./day), but these later showed evidence of endocrine imbalance suggestive of Cushing's syndrome. She also mentions, however, a case of Cushing's syndrome without hypertension in which there was excessive glucocorticoid excretion.

Apart from those cases with evidence of endocrine disturbance, it was thought that there might be some correlation between a disturbance of adrenal cortical function and the different clinical types of hypertensive disease. For example, the occurrence of toxemia of pregnancy followed by persistent hypertension would suggest some disturbance of adrenal cortical function. However, no evidence to support this possibility has been found. It was also thought that there might be a correlation between a disturbance of adrenal cortical function and the more severe form of hypertensive disease—the malignant hypertension syndrome. Here again the results were negative.

2. *Excessive production of salt-active corticoids.* In the light of the observations of the pressor effects of desoxycorticosterone acetate and salt in man and animals, an excessive elaboration of steroids having similar effects would appear to be a possible factor in human hypertension. As already pointed out, investigation of this possibility is hampered by lack of a sufficiently sensitive specific technique for estimation of these steroids.

We have some indirect evidence, however, which would suggest that there is no gross excess of steroid elaboration by the adrenal cortex in hypertension. If there were such a gross excess of steroid production by the adrenal cortex, some increase in 17-ketosteroid production would be expected, as these steroids probably include the metabolites of the adrenal steroids (37). The findings in this series have, however, been normal, and in a previous series (22) the results were slightly lower than normal.

Cheng and Sayers (35) have recently adduced evidence that excessive desoxycorticosterone acetate administration produces a condition of hypocorticism characterized by a deficiency of glucocorticoids, with increased

sensitivity to insulin. There is certainly no evidence of a deficiency of glucocorticoid production in any type of hypertension, from either this investigation or that of Selye (15); nor is there an obviously increased sensitivity to insulin in hypertension, as the results of Mirsky *et al.* (17) with the insulin tolerance test indicate.

Corcoran *et al.* (20) using a chemical (formaldehydogenic) technique found, however, that slight "hypercorticoiduria" was disclosed by repeated estimations in any one case. They found that 20 out of 46 cases showed "hypercorticoiduria" with a single estimation, whereas 9 out of 10 cases showed it with four or more estimations (although 7/10 of the means of all their replicates will be seen to be within normal limits). They suggest that "undue lability of corticoid excretion is commonly part of the biochemical pattern of the disease, and may be associated with other evidence of vasomotor and sympathetic instability which are common in hypertension." Tobian and Joseph (19), using a similar technique for estimations in plasma, found no difference from normal and concluded that marked adrenal hyperfunction was unlikely.

Evidence to support an excessive elaboration of salt-active steroids from the adrenal cortex in human hypertension has also been sought by study of the sodium balance (14) and sodium content of body fluids (15, 16). Perera (2) considers that there is convincing evidence for a disturbance of salt and water metabolism associated with hypertension. But it would appear, as Sayers (36) points out, that the reduction in blood pressure which follows salt restriction in hypertension is not great enough to implicate a salt-active steroid as an etiologic agent, when compared with the known effects of salt restriction upon the hypertensive action of desoxycorticosterone in experimental animals and man.

3. *Relative deficiency of glucocorticoid production.* The observations of Selye (7) and Woodbury *et al.* (8) in animals and Perera and Pines (11) in man, that the pressor effects of desoxycorticosterone administration can be inhibited by simultaneous administration of glucocorticoids, whole adrenal cortical extract, or ACTH, raise the possibility that there may be a deficiency in glucocorticoid production by the adrenal cortex rather than an excessive production of salt-active steroids. Selye (4) has pointed out that the ratio of glucocorticoid to "mineralocorticoid" production may be more important than the absolute level of either.

Our findings and those of F. L. Selye (15), that the glucocorticoid output is certainly not lowered in hypertension, suggest that the ratio is not disturbed, unless there is a considerable excess of salt-active steroids being produced. This has already been considered to be unlikely.

Furthermore, it must be remembered that the only steroids which have

been isolated from the body fluids are the glucocorticoids, compound E and compound F (11-dehydro-17-hydroxycorticosterone and 17-hydroxycorticosterone) (37, 38, 39). These are, therefore, the only steroids known to be produced by the adrenal cortex under physiologic or pathologic conditions. It seems likely that the well-known adrenal cortical effects on electrolyte balance could be exerted by glucocorticoids. Woodbury *et al.* (40) have reached the tentative conclusion that glucocorticoids (in common with whole adrenal cortical extract and ACTH) "can 'normalize' plasma sodium regardless of the direction of the deviation from normal," in marked contrast to desoxycorticosterone. All the effects of ACTH stimulation of the adrenal cortex have been reproduced by administration of 17-hydroxycorticosterone (41).

These findings then, would suggest that the best indication of adrenal cortical function can be given by glucocorticoid estimation, as was in fact carried out in this investigation.

Our conclusion, therefore, must be that there is no evidence from our investigation for a significant disturbance of adrenal cortical function in hypertension. It must be admitted that the techniques available for the determination of adrenal cortical function are by no means as precise as could be desired; but at present it would appear that, although the adrenal cortex is necessary for the maintenance of blood pressure, it is not a primary factor in the etiology of either renal or "essential" hypertension.

## SUMMARY AND CONCLUSIONS

Adrenal cortical function has been investigated in various clinical types of hypertension, using as indications the urinary excretion of glucocorticoid determined by biologic assay and of 17-ketosteroids determined chemically.

Normal results have been obtained, apart from a case of Cushing's syndrome due to pituitary basophilism.

The significance of the findings is discussed in the light of relevant experimental and clinical observations. It is concluded that there is at present no direct evidence for a significant disturbance of adrenal cortical function in renal or "essential" hypertension.

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# THE ACTION OF ESTROGEN AND PROGESTERONE ON THE GONADOTROPIN CONTENT OF THE PITUITARY OF THE MONKEY (*MACACA MULATTA*)*

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IN a recent review, Greep and Jones (1) indicated that though a great deal is known concerning the action of the pituitary hormone on the target organs, very little is known "as to what the secretory products of the target organs do to the pituitary gland." It is universally accepted now that the ovary is under the control of three gonadotropins from the anterior pituitary. These are the follicle-stimulating hormone (FSH), the luteinizing hormone (LH) and the luteotropic hormone (LTH). There is no doubt that the gonadotropins are in turn regulated by the sex steroids but their specific role in regulating the hypophysis is still questionable.

A marked increase in the gonadotropin concentration of the hypophysis after castration has been demonstrated in the guinea pig (2), the rabbit (3) and the rat (4). In 1932, Zondek (5) reported an increase in the gonadotropin concentration of the urine of castrated men and women.

The results obtained from treatment with estrogenic substances have been somewhat more controversial. Numerous investigators have indicated that estrogens stimulate the release of LH (6, 7, 8) while suppressing FSH production (9, 10, 4, 11). The injection of large doses of estrogen in the human causes a definite decrease in the excretion of gonadotropins (12), as well as a decrease in pituitary potency (13). On the other hand, moderate doses of estrogen do not lower the postmenopausal excretion of urinary gonadotropins in women (14) nor do they suppress urinary gonadotropins in castrated women (15). From these and other data, Junck, Heller and Nelson (16) concluded that moderate amounts of estrogen exert little inhibiting action on the pituitary gland but large "unphysiological" amounts of estrogen are potent inhibitors. In contradistinction to this, Greep and Jones (11) indicate that smaller amounts of estrogen will coun-

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teract the effect of castration on the gonad-stimulating potency of the pituitary gland. Byrnes and Meyer (17) have arrived at the same conclusion from experiments involving parabiosis.

Data on the role of progesterone in regulating the concentration and release of gonadotropins in the pituitary gland are still inadequate. Herlant (18, 19) reported a decrease in the gonadotropic potency of the pituitary gland of rats treated with progesterone, but Fevold (20) and Greep and Jones (11) have indicated that progesterone had no effect.

In the course of studies on the steroid regulation of uterine growth, pituitaries from monkeys treated with estradiol, progesterone and a combination of both steroids have been analyzed for gonadotropins. In addition, analyses are reported of pituitaries from animals treated with a folic acid antagonist.

## MATERIAL AND METHODS

The monkeys (*M. mulatta*) used in these experiments were healthy, adolescent or mature females weighing between 3.5 and 5.5 kilograms. On arrival from a commercial dealer,[1] the animals were maintained in isolation for a period of at least one month before being placed in the experimental colony. All monkeys were kept in air-conditioned rooms, thermostatically controlled as to temperature and humidity, and maintained on a standard diet of fruits, vegetables and Rockland Farm monkey pellets.

The injections were started on the day of castration and the monkeys were killed with nembutal twenty-four hours after the last injection. At autopsy, the pituitaries were removed, weighed on a torsion balance and placed in a deep-freeze. All hormones were injected subcutaneously in 0.1 ml. of sesame oil, except for the 20-mg. dose of progesterone[2] which was administered in 1/3 ml. of propylene glycol warmed to 37° C. Injections were made daily at approximately the same time. None of the monkeys menstruated during the course of these experiments. In most of the treatments the estradiol[2] was administered in daily doses of 10 micrograms and progesterone in a daily dose of 2 milligrams. To study the effects of higher doses, 100 μg. of estradiol and/or 20 mg. of progesterone were given in several instances.

In a second series of experiments, the effects of a folic acid antagonist (4-aminopteroylglutamic acid[3]) on estrogen and progesterone action were

[1] Obtained from the Chase Animal Farm, Egypt, Massachusetts.

[2] Obtained through the courtesy of the Schering Corporation, Bloomfield, New Jersey.

[3] Obtained through the courtesy of Dr. E. L. R. Stokstad, Lederle Laboratories, Pearl River, New York.

studied. Castrated monkeys were given daily injections of aminopterin along with the hormones, until they menstruated or became moribund. At this time, autopsies were performed and the pituitaries removed for assay.

Gonadotropin assays were performed in accordance with the method of Zarrow and Salter (21). The pituitary was weighed upon removal from the sella turcica and immediately placed in a deep-freeze. For assay, the pituitary was homogenized in saline and injected into 21-day-old or 22-day-old female rats of the Harvard-Wistar strain. The homogenate was diluted, so that logarithmically increasing concentrations were injected. Injections were made twice daily for four days and autopsies were performed on the fifth day. The ovaries and uteri were dissected out, the uteri split open, blotted on bibulus paper to remove excess moisture, and weighed on a Roller-Smith torsion balance to the nearest 0.1 mg. The ovaries were fixed in Bouin's solution and prepared for histologic examination, in order to determine the character of the response. In no case, was a pituitary assayed after more than seven days in the deep-freeze.

In calculating gonadotropic potency, the ovarian and uterine weights of the assay animals were plotted against the various doses of the pituitary homogenate, and regression lines were drawn through these points. The amount of homogenate causing 100 per cent increase in uterine weight was considered to contain one gonadotropic unit (G.U.); and the concentration was reported both on the basis of the entire pituitary and per 100 mg. of gland weight.

TABLE 1. THE EFFECT OF ESTRADIOL ON THE GONADOTROPIN CONTENT OF THE PITUITARY OF THE CASTRATED MONKEY

| Monkey No. | Dose of estradiol, μg./day | Length of treatment (days) | Body wt., Gm. | Pituitary | | Gonadotropin | |
|---|---|---|---|---|---|---|---|
| | | | | Wt., mg. | mg. % of body wt. | in entire pit. | in 100 mg. of pit. |
| 163 | — | 600 | 5,430 | 71.2 | 1.3 | 101* | 141 |
| 165 | — | 645 | 5,300 | 68.7 | 1.3 | 76** | 110 |
| 147 | 10 | 28 | 3,240 | 82.4 | 1.5 | 63 | 77 |
| 156† | 10 | 270 | 3,854 | 64.9 | 1.7 | 63 | 100 |
| 173 | 10 | 300 | 4,175 | 86.0 | 2.1 | 66 | 76 |
| 190 | 100 | 185 | 3,263 | 64.9 | 2.0 | 0‡ | 0 |

* Assayed 26.8 mg. of the pituitary.
** Assayed 27.3 mg. of the pituitary.
† One injection of progesterone.
‡ Assayed 44.2 mg. of the pituitary.

## RESULTS

Examination of the data in Table 1 shows a high level of gonadotropin in the pituitary of the castrated animal. Pituitaries from monkeys #163 and #165 contained 141 and 110 gonadotropin units (G.U.) per 100 mg. of gland. These animals had been castrated for 600 and 645 days respectively and had received no treatment throughout this period. Daily injections of 10 μg. of estradiol for 270 and 300 days in 2 castrated monkeys (#156 and #173) failed to produce any marked change in gonadotropic activity, although a slight decrease was observed. The pituitary of monkey #156 contained 100 G.U. and that of monkey #173 contained 76 G.U. per 100 mg. of gland. Furthermore, prolonged treatment with progesterone both in a low dose of 2 mg. daily and a high dose of 20 mg. daily for periods of from 12 to 312 days did not result in any marked change in the gonadotropic potency of the hypophysis (Table 2).

TABLE 2. THE EFFECT OF PROGESTERONE ON THE GONADOTROPIN CONTENT OF THE PITUITARY OF THE CASTRATED MONKEY

| Monkey No. | Dose of progesterone, mg./day | | Body wt., Gm. | Pituitary | | Gonadotropin | |
|---|---|---|---|---|---|---|---|
| | | | | Wt., mg. | mg. % of body wt. | in entire pit. | in 100 mg. of pit. |
| 155 | 2 | 312 | 7,470 | 100.5 | 1.3 | 67 | 67 |
| 195 | 20 | 12 | 3,967 | 82.9 | 2.1 | 98 | 118 |
| 196 | 20 | 183 | 4,153 | 59.1 | 1.4 | 53* | 89 |

* Assayed 31.8 mg. of the pituitary.

On the other hand, the pituitary from monkey #190 which was treated with 100 μg. of estradiol daily for 185 days, was entirely depleted of gonadotropins (Table 1). The only other group of monkeys whose pituitaries were depleted of gonadotropins were those treated with both estradiol and progesterone (Table 3). Thus, the pituitaries of 3 monkeys treated with daily injections of 10 μg. of estradiol and 2 mg. of progesterone for 200 to 308 days elicited no response on assay in the immature rat. In one instance, only 20 days of treatment was sufficient to deplete the pituitary of its gonadotropins (monkey #203; Table 3). As could be expected, higher doses of estrogen and progesterone gave similar results. Two monkeys (#192 and #193) injected with 100 μg. of estradiol and 20 mg. of progesterone for from 85 to 181 days yielded pituitary glands that were completely devoid of gonadotropins. On the other hand, lowering the daily dose of progesterone to 0.5 mg. failed to deplete the pituitary entirely of its gonadotropins. After 211 days of treatment with 10 μg. of

estradiol and 0.5 mg. of progesterone, the pituitary gland of monkey #188 (Table 3) was found to contain 12 G.U.

Of obvious importance in this study is the time required for the pituitary gland to lose or regain its activity. For example monkey #150 (Table 4) was treated with estrogen for 383 days and at this time probably had a high gonadotropin content, since it was comparable with monkeys #156

TABLE 3. THE EFFECT OF ESTROGEN AND PROGESTERONE ON THE GONADOTROPIN CONTENT OF THE PITUITARY OF THE CASTRATED MONKEY

| Monkey No. | Estrogen, μg./day | Progesterone, mg./day | Length of treatment (days) | Body wt. (Gm.) | Pituitary | | Gonadotropin | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Wt., mg. | mg. % of body wt. | in entire pit. | in 100 mg. of pit. |
| 188 | 10 | 0.5 | 211 | 4,790 | 71.5 | 1.5 | 12 | 17 |
| 203 | 10 | 2 | 20 | 3,215 | 72.9 | 2.2 | neg. | neg. |
| 166 | 10 | 2 | 200 | 4,100 | 77.2 | 1.9 | neg. | neg. |
| 151 | 10 | 2 | 308 | 6,925 | 39.5 | 0.5 | neg. | neg. |
| 186 | 10 | 2 | 291 | 5,700 | 72.3 | 1.3 | neg. | neg. |
| 193 | 100 | 20 | 181 | 3,708 | 59.1 | 1.6 | neg. | neg. |
| 192 | 100 | 20 | 85 | 4,805 | 61.3 | 1.3 | neg. | neg. |

and #173 (Table 1). However, after further treatment with 10 μg. of estradiol and 2 mg. of progesterone for 19 days, the gonadotropic potency was negligible. Similarly, when monkey #171 was treated with 2 mg. of progesterone for 275 days and then injected with both 10 μg. of estradiol and 2 mg. of progesterone for 20 days, the pituitary was depleted of its gonadotropins (Table 4). Furthermore, pretreatment with 1 μg. of estradiol and 2 mg. of progesterone for 208 days, followed by 10 μg. of estradiol and 2 mg. of progesterone for 20 days, resulted in a negative response, whereas the same pretreatment followed by the injection of only estradiol for 20 days resulted in an accumulation of gonadotropins as high as that of the untreated castrate controls (monkeys #189 and #191, Table 4). These data demonstrate that within 20 days after injections of both estradiol and progesterone, the pituitary had lost its gonadotropins.

On the other hand, approximately 20 days after the withdrawal of either estrogen or progesterone, the gonadotropic hormones began to reaccumulate in the pituitary gland. It can be seen from the data on monkeys #153, #170 and #185 (Table 4) that the gonadotropic potency of the pituitary is increased after having been depleted by combined treatments. However, the amount of gonadotropins which accumulated under the influence of estrogen is less and the rate is slower than that of the depletion caused by the combined action of the steroids.

TABLE 4. THE EFFECT OF VARIOUS ESTROGEN AND PROGESTERONE TREATMENTS ON THE GONADOTROPIN CONTENT OF THE PITUITARY OF THE CASTRATED MONKEY

| Monkey No. | Treatment | | | | Subsequent treatment | | Body wt., Gm. | Pituitary | | Gonadotropin | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Estrogen | | Progesterone | | | | | Wt., mg. | mg. % of body wt. | in entire pit. | in 100 mg. of pit. |
| | μg. | days | mg. | days | dose | days | | | | | |
| 150 | 2–50 | 383 | — | — | 10 μg. est. 2 mg. prog. | 19 | 8,780 | 85.8 | 1.0 | 0 | 0 |
| 168 | — | — | 2 | 302 | 10 μg. est. | 20 | 5,460 | 86.2 | 1.6 | 70 | 81 |
| 171 | — | — | 2 | 275 | 10 μg. est. 2 mg. prog. | 20 | 5,505 | 72.8 | 1.3 | 5 | 7 |
| 185 | 10 | 218 | 2 | 218 | 10 μg. est. | 20 | 4,415 | 52.8 | 1.2 | 20* | 38 |
| 153 | 10 | 307 | 2 | 307 | 10 μg. est. | 20 | 6,367 | 58.3 | 0.9 | 97 | 166 |
| 170 | 10 | 275 | 2 | 275 | 10 μg. est. | 21 | 5,282 | 86.0 | 1.6 | 12 | 14 |
| 191 | 1 | 208 | 2 | 208 | 10 μg. est. | 20 | 4,600 | 75.8 | 1.6 | 108** | 143 |
| 189 | 1 | 208 | 2 | 208 | 10 μg. est. 2 mg. prog. | 20 | 4,120 | 54.8 | 1.3 | 6† | 11 |

* Assayed 34.1 mg. of the pituitary.
** Assayed 40.3 mg. of the pituitary.
† Assayed 27.3 mg. of the pituitary.

Aminopterin treatment caused a loss of gonadotropins in all but 2 of the monkeys (#157 and #159) and only 1 of these (monkey #157) showed a normal gonadotropin concentration of 124 G.U. per 100 mg. of gland (Table 5). It is of interest to note that these 2 monkeys (#157 and #195) showed the smallest drop in body weight in the group. It is possible that part of this effect may be due to inanition but the age factor must also be considered, as these monkeys were relatively young.

Histologic examination of the ovaries of the assay rats gave some indication of the type of gonadotropin present in the pituitary glands of the monkeys. Although lack of corpora lutea would not entirely rule out the presence of LH, their absence would indicate that the major activity of the glands was of the FSH type. This type of reaction, *i.e.*, follicle stimulation, was seen consistently in all the ovaries except in the group of rats treated

with pituitary homogenates from monkey #157. In the latter instance, the ovaries showed many corpora lutea. Uterine stimulation was noted in all the assay rats, indicating the release of estrogen, hence the presence of some LH (22). It is obvious, however, from an examination of the ovaries that only minute amounts of LH were present; and since the rats were not hypophysectomized, it is difficult to state whether the LH was present in the homogenate or came from the pituitary of the assay rat. However,

TABLE 5. GONADOTROPIN CONTENT OF THE PITUITARY OF CASTRATED MONKEYS TREATED WITH FOLIC ACID ANALOGUES

| Monkey | Treatment | Body wt., Gm. | Pit. wt., mg. | $\frac{\text{Pituitary, mg.}}{\text{Body, Gm.}} \times 100$ | Gonadotropin | |
|---|---|---|---|---|---|---|
| | | | | | in entire pit. | in 100 mg. of pit. |
| 181 | Estradiol & FAA* | 2,327 | 53.7 | 2.3 | neg. | neg. |
| 176 | Estradiol & Progest. & FAA | 3,160 | 65.5 | 2.1 | neg. | neg. |
| 178 | Progesterone & FAA | 3,078 | 66.1 | 2.1 | neg. | neg. |
| 157 | Estradiol & FAA | 5,454 | 83.6 | 1.5 | 104 | 124 |
| 175 | Estradiol & FAA | 2,315 | 62.5 | 2.1 | neg. | neg. |
| 177 | Estradiol, Progesterone & FAA | 2,700 | 56.3 | 2.1 | neg. | neg. |
| 159 | Estradiol & FAA-IV | 4,050 | 85.2 | 2.1 | 10 | 12 |
| 179 | Estradiol & FAA | 2,991 | 52.3 | 1.7 | neg. | neg. |

* Folic acid antagonist.

when the pituitary from monkey #194 was assayed in both intact and hypophysectomized rats, a small amount of luteinizing hormone was found to be present.

Estrogen tended to increase the weight of the pituitary when compared as a function of body weight (Table 1), whereas progesterone or progesterone and estrogen had little effect (Tables 2 and 3). The pituitaries of the monkeys treated with aminopterin and the steroids also tended to increase in weight, though this might have been due to the loss in body weight produced by the folic acid antagonist.

## DISCUSSION

These results indicate that moderate doses of estrogen have no effect on the concentration of gonadotropins in the pituitary of the monkey, whereas large doses cause a definite and marked depletion. Junck, Heller and Nelson (16) have reported similar results in the rat, though other

investigators have stated that lower doses of estrogen can inhibit the secretion of gonadotropins by the pituitary (1, 17).

Progesterone alone in either moderate or large doses had no effect on the gonadotropin content of the pituitary gland. But it is significant that treatment with moderate doses of estrogen and progesterone, neither of which was effective when given alone, produced a depletion of gonadotropins in the pituitary when given together. Furthermore, this result could be obtained within twenty days. It is difficult to compare these data with those obtained by other investigators. Differences in design of experiment, age and species used, length of treatment and dosage become paramount. Bradbury (23) observed that the action of progesterone in an estrogen-treated rat was to prevent the discharge of gonadotropin, whereas Everett (24) has shown that the simultaneous injection of both steroids stimulates ovulation. Part of the explanation may be due to the fact that these two steroids may have a synergistic or antagonistic action, depending on the ratio of hormones injected and the end organs examined (24). It would appear that such a relationship exists also for the pituitary gland.

In view of these different effects of the steroids on the pituitary gland it is possible to speculate on their physiologic action. During pregnancy the increased secretion of the steroids would tend to inhibit the pituitary gland. If this action requires about twenty days, it would coincide with the secretion of chorionic gonadotropin, which reaches a maximal concentration in the blood of the monkey about twenty days after implantation of the ovum (26). Thus, as the pituitary is blocked, the placenta assumes its luteotropic function (27). The presence of the progestational hormone in the follicular phase of the cycle (28, 29) may indicate a possible role in ovulation, according to the short-term action indicated by Everett (24). A better understanding of these relationships in pregnancy and the menstrual cycle must await more precise methods for measuring each of the gonadotropins both qualitatively and quantitatively.

All animals in these experiments, with the exception of those treated with folic acid analogues, were in excellent health and maintained their body weights throughout the course of treatment. The monkeys that were treated with aminopterin lost weight rapidly and became anemic. With 2 exceptions, all the aminopterin-treated monkeys showed a depletion of gonadotropin activity from the pituitary gland and in only 1 of these was any appreciable amount found. Whether this loss was due to inanition from the folic acid deficiency or to the relative immaturity of the animals is still questionable. Although some investigators have reported a loss of gonadotropins on starvation (30, 31), others have reported no change (32, 33, 34) or even an increase (32, 33, 35, 36). Recently both Maddock and Heller (37) and Meites and Reed (38) have described a decrease in

circulating gonadotropin in rats on inanition diets, but no change in the gonadotropic potency of the pituitary gland.

SUMMARY

Daily treatment with either 10 micrograms of estradiol or from 2 to 20 milligrams of progesterone failed to affect the concentration of gonadotropins in the pituitary gland of the castrated monkey. Treatment with 100 micrograms of estradiol alone, however, or simultaneous treatment with 10 micrograms of estradiol and 2 milligrams of progesterone resulted in a complete loss of gonadotropins from the pituitary gland. Cessation of treatment permitted the reaccumulation of gonadotropins in the hypophysis within twenty days. A marked depletion of pituitary gonadotropins was noted after treatment with aminopterin and the steroids. The possible relationship of the sex steroids in the regulation of pituitary function during pregnancy and the menstrual cycle is discussed.

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# THE EFFECTS OF CHORIONIC GONADOTROPIN AND PROLACTIN ON THE MAINTENANCE OF CORPUS LUTEUM FUNCTION*†

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IN RECENT years increased importance has been attached to corpus luteum dysfunctions as causes of habitual abortion, sterility and menorrhagia (1). The reported investigations to correct corpus luteum disturbances in humans in a direct physiologic manner with luteinizing or luteotropic gonadotropins have been indeterminate and led to the present study. After Aschheim and Zondek had reported in 1927 that human chorionic gonadotropin induced follicular maturation and corpus luteum formation in rats and mice (2), the hormone was used extensively in the human as a luteinizing agent, most commonly in instances of functional uterine bleeding. The initial enthusiasm (3) waned gradually as further reports (4) of the responses in humans and monkeys following such therapy provided little evidence to support the therapeutic value of chorionic gonadotropin in ovarian dysfunctions. Since then, references to such therapy in the literature have, in the main, been limited to reviews and editorials which have questioned the value of chorionic gonadotropic therapy in women (5, 6). As recently as 1949 one reviewer considered any therapeutic responses to the drug to be due to the psychic effect of the injections and suggested, as had others (7–9), that failure of response might be due to an inadequacy of the usual clinical dosages of 500 and 1,000 I.U. Hamblen and Ross (8) have postulated that the effective pharmacologic dose in the human is probably in excess of 8,000 units daily. Hisaw (10) reported a limited prolongation of corpus luteum function in the monkey with the use of large daily doses (1,000 I.U.) of chorionic gonadotropin. Since large dosages of the commercial products were bulky and had caused severe reactions in the human (9, 11, 12), similar investigations in women were not possible until Brown and Bradbury's (11) recent use of

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a special chorionic gonadotropin preparation.[1] This product prolonged the life span of corpora lutea, as evidenced by menstrual histories, endometrial tissue studies, urine pregnanediol assays, and the finding of corpora lutea at laparotomy. In 2 cases, the daily administration of 20,000 I.U. of the hormone, the largest amount used, produced menstrual delays of twelve and nineteen days so long as the injections were continued, and in 1 patient, 5,000 I.U., the minimal effective amount, deferred menses for six days. A fourth patient received 10,000 I.U. of the hormone and noted a menstrual delay of ten days. Doses of 2,500 I.U. were ineffectual. The action was considered to be luteotropic, since it was limited to the maintenance of the corpus luteum. Since then de Watteville (12) similarly has used 20,000 I.U. of chorionic gonadotropin with analogous corpus luteum effects in 3 patients, but reported severe general and local toxic reactions in all cases. The luteotropic doses advised by Brown and Bradbury (11) have not been used generally, a) because their special preparation is not generally available, and b) because of the relatively high cost of such dosages and toxic reactions to them, when available commercial products are used.

The investigations with chorionic gonadotropin have been paralleled more recently by studies on the luteotropic properties of lactogenic hormone. The demonstration by Astwood (13) in 1941 and by others.(14) later of a luteotropic function for the lactogenic hormone in the rat led to the postulate that in the human, in addition to a pituitary luteinizing hormone which luteinizes the ovarian granulosa cells, the lactogenic hormone may serve as a third pituitary gonadotropic substance, luteotropin, which regulates the activity and maintains the function of the luteal cells (15, 16). The findings in animals were followed by reports (17, 18) of the successful use of the hormone in cases of menorrhagia and threatened abortion. Kupperman and Fried (18) in 1943 questioned a luteotropic response in their successfully treated cases, since they found that similar or increased dosage schedules in normal cyclic women did not produce any change in the length of the menstrual cycles, in the menstrual flow, or in the normal endometrial changes. More recently the failure of Bradbury and his colleagues (19) to influence the menstrual cycle in normal women with large amounts of the lactogenic hormone likewise led them to doubt the luteotropic capacity of the hormone in the human. The first objective evidence in support of any lactogenic hormone-corpus luteum relationship in the human has been the very recent demonstration by Segaloff (20)

[1] Chorionic gonadotropin, as a dried powder, was dissolved in a phosphate buffer (pH 7.4), sterilized by passing through a Jena filter, and used without a preservative in a dilution of 5,000 I.U. per milliliter.

of a cyclic urinary prolactin pattern in women, with a rise during the phase of increased corpus luteum function.

The purpose of this study was to determine the effects of a combination of chorionic gonadotropin and lactogenic hormone (luteotropin) on corpus luteum function in women with normal cyclic menses. The use of such a combination of these hormones is based on the theory of a synergistic action of the pituitary luteinizing and lactogenic hormones during the normal menstrual cycle. The theory of the synergistic mechanism is supported by the experiments of Everett (21), who demonstrated that in rats cholesterol, the precursor of progesterone, was dependent upon the luteinizing hormone for its accumulation in luteal cells and upon the luteotropic hormone for its transformation to progesterone by the luteal cells. He also found that maintenance of normal corpus luteum secretion was subject to a proper ratio of luteinizing and luteotropic hormones.

## PROCEDURE

The 12 cases selected for investigation included 8 normally menstruating sterile patients, 2 castrates, and 2 patients with menstrual dysfunction, *i.e.*, one with hypomenorrhea and the other with menorrhagia. The corpus luteum activity of the 10 menstruating patients was studied over a period of three or more months previous to therapy and during 28 courses of treatment. The investigations included the examination of endometrial biopsy specimens during various phases of the menstrual cycle, the evaluation of the daily waking temperature records, and urine pregnanediol assays.[2] The endometrium[3] mirrored the corpus luteum effect since the last bleeding episode. The waking temperature record provided day-to-day evidence of corpus luteum function, since the postovulatory temperature elevation in normal women has been demonstrated to be due to progesterone and has been correlated with the duration of the functional phase of the corpus luteum. In the absence of pregnancy, a temperature elevation that is prolonged beyond sixteen days has been considered to be indicative of continued progesterone production by a persistent functional corpus luteum (23).

The hormones used in this study consisted of sheep lactogenic hormone[4] and chorionic gonadotropin.[5] The latter has a luteinizing effect similar to the pituitary luteinizing hormone and is readily available.

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[2] Colorimetric method of Sommerville and Marrian (22).

[3] Stained for glycogen with Best's carmine.

[4] Luteotropin, which was generously supplied through the courtesy of E. R. Squibb & Sons.

[5] Follutein and A.P.L. which were generously supplied, respectively, by E. R. Squibb & Sons and Ayerst, McKenna & Harrison.

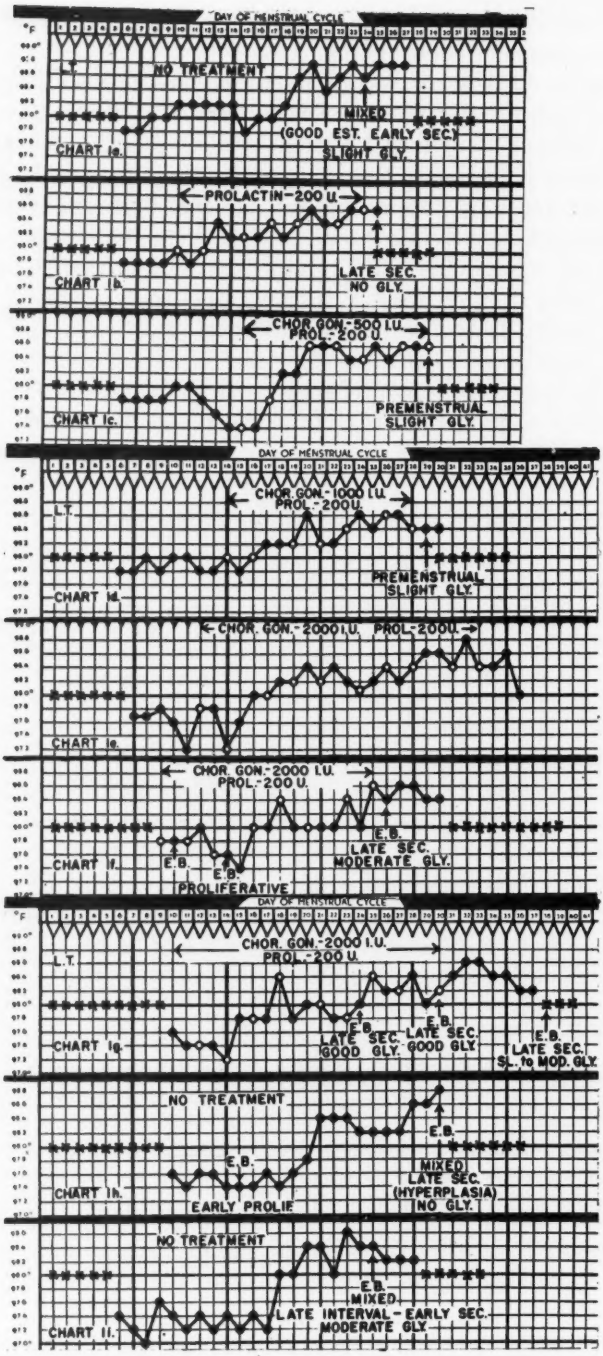
DAY OF MENSTRUAL CYCLE
°F
L.T.
NO TREATMENT
MIXED
(GOOD EST. EARLY SEC.)
SLIGHT GLY.
CHART Ia.
PROLACTIN-200 U.
LATE SEC.
NO GLY.
CHART Ib.
CHOR. GON.-500 I.U.
PROL.-200 U.
PREMENSTRUAL
SLIGHT GLY.
CHART Ic.
DAY OF MENSTRUAL CYCLE
°F
CHOR. GON.-1000 I.U.
PROL.-200U.
L.T.
PREMENSTRUAL
SLIGHT GLY.
CHART Id.
CHOR. GON.-2000 I.U. PROL-200U.
CHART Ie.
CHOR. GON.-2000 I.U.
PROL.-200 U.
E.B.
LATE SEC.
MODERATE GLY.
CHART If.
E.B.
E.B.
PROLIFERATIVE
DAY OF MENSTRUAL CYCLE
°F
CHOR. GON.-2000 I.U.
PROL.-200 U.
L.T.
E.B.
LATE SEC.
GOOD GLY.
E.B.
LATE SEC.
GOOD GLY
E.B.
LATE SEC.
SL. to MOD. GLY.
CHART Ig.
NO TREATMENT
E.B.
E.B.
MIXED
LATE SEC.
(HYPERPLASIA)
NO GLY.
CHART Ih.
EARLY PROLIE
NO TREATMENT
E.B.
MIXED
LATE INTERVAL - EARLY SEC.
MODERATE GLY
CHART Ii.

FIGURE 1

OBSERVATIONS

In this study it was found that the minimal effective combination of hormones that caused prolongation of corpus luteum function in 4 patients during 7 courses of treatment consisted of 2,000 I.U. of chorionic gonadotropin and 200 units of lactogenic hormone (Figs. 1 (e, f, g); 2 (b); 3 (b, d); 4 (b)) since preliminary studies (Figs. 1 (b, c, d); 2 (d, e): 3 (f); 5 (b); Patients H.N., G.B. and A.B.) indicated that there was no effect upon the

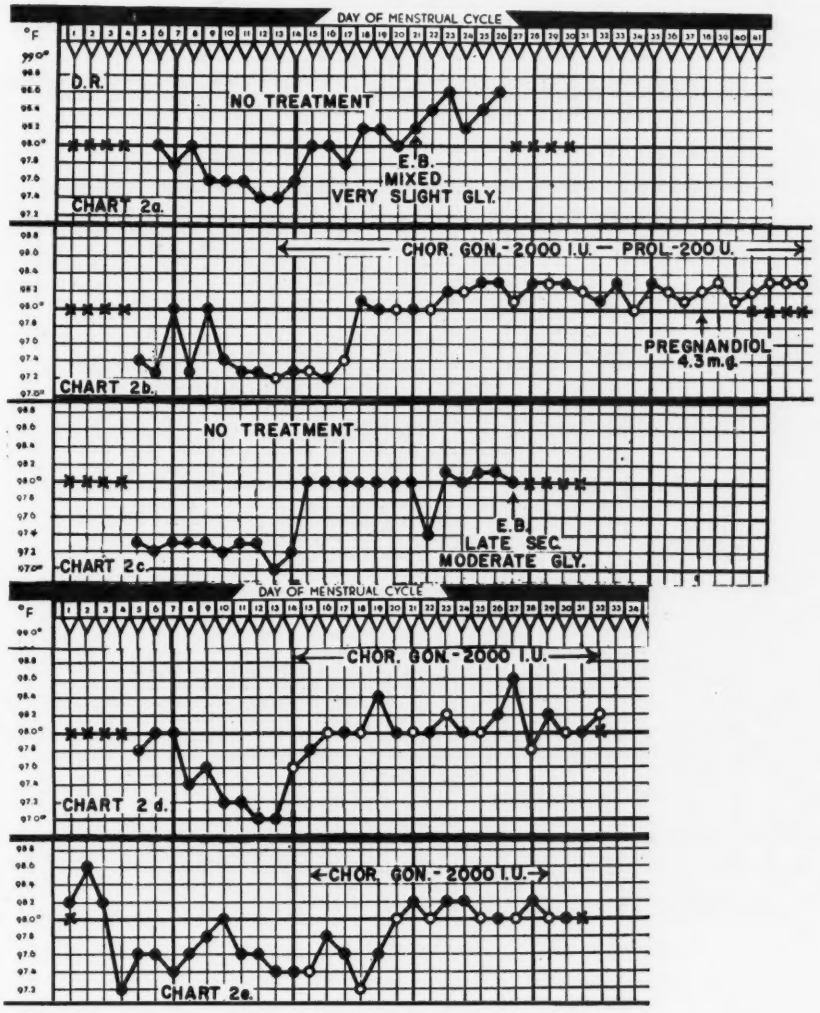


FIGURE 2

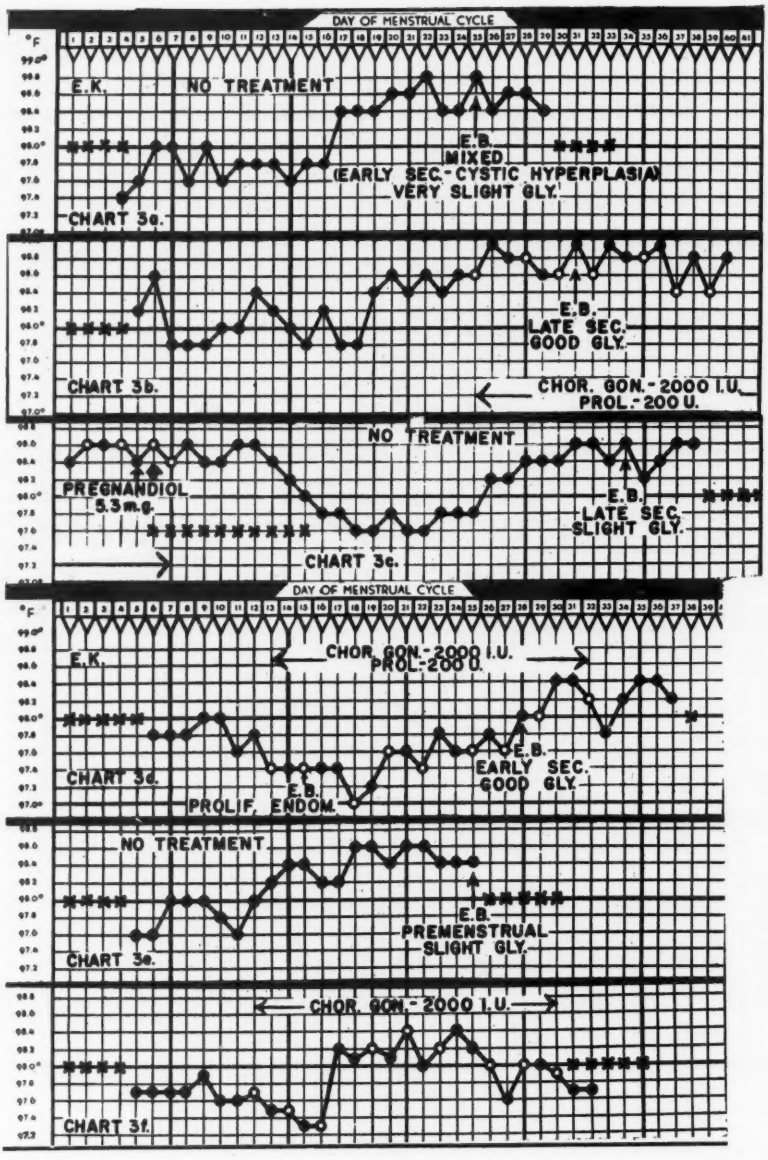
DAY OF MENSTRUAL CYCLE
°F
E.K.
NO TREATMENT
E.B.
MIXED
(EARLY SEC.-CYSTIC HYPERPLASIA)
VERY SLIGHT GLY.
CHART 3a.
E.B.
LATE SEC.
GOOD GLY.
CHART 3b.
CHOR. GON.-2000 I.U.
PROL.-200 U.
NO TREATMENT
PREGNANDIOL
5.3 m.g.
E.B.
LATE SEC.
SLIGHT GLY.
CHART 3c.
DAY OF MENSTRUAL CYCLE
°F
E.K.
CHOR. GON.-2000 I.U.
PROL.-200 U.
E.B.
EARLY SEC.
GOOD GLY.
CHART 3d.
E.B.
PROLIF. ENDOM.
NO TREATMENT
E.B.
PREMENSTRUAL
SLIGHT GLY.
CHART 3e.
CHOR. GON.-2000 I.U.
CHART 3f.

FIGURE 3

corpus luteum by similar or smaller doses of either hormone alone or by combinations of lesser amounts. The hormones were given three times a week and were started within a week following the ovulatory temperature rise. The period of maintained corpus luteum function depended upon the duration of therapy. When therapy was stopped three days before or at the expected date of menses, the onset of the menstrual flow was delayed for three and six days, respectively (Figs. 1 (f); 3 (d)). When injections were continued for three, seven and nine days beyond the expected date of menses, there were menstrual delays of eleven days (Figs.

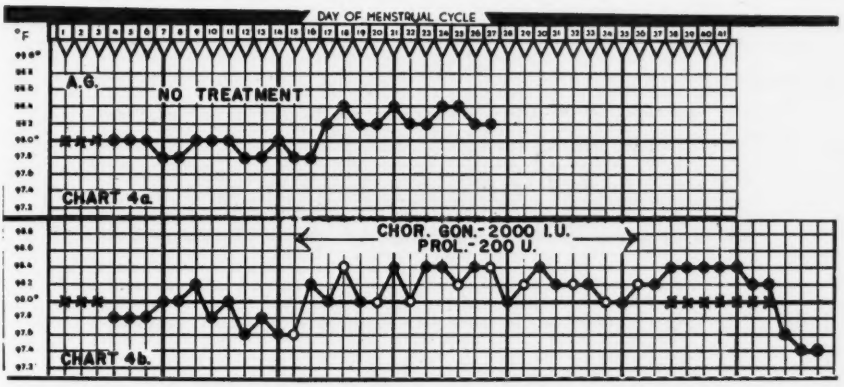


FIGURE 4

1 (e, g); 4 (b)). Uninterrupted therapy delayed menstrual bleeding for thirteen days (Figs. 2 (b); 3 (b)). An increased frequency of injections did not further affect the results (Fig. 2 b). Associated with each menstrual delay was a prolongation of the post-ovulatory temperature elevation, a premenstrual endometrium of the late secretory (progesteronic) type with the presence of glycogen, and an increase in duration and amount of menstrual flow. In the 2 cases tested, urinary pregnanediol levels (4.3 and 5.3 mg./24 hrs.) were maintained until the onset of bleeding. During therapy, all premenstrual endometria were normal, in contrast to the control group of endometria, which frequently were of the "mixed" type (estrogenic-progesteronic) or of the secretory type with glycopenia, suggesting an improvement in corpus luteum activity. It is not only pertinent that moderate amounts of glycogen were present in all endometria during therapy, but that the glycogen content was maintained late in the cycle in moderate amounts (Figs. 1(f, g); 3(b)) and until the onset of menses (Fig. 1g). The effect of therapy was demonstrable only during the menstrual cycle in which the hormones were used, since in the subsequent

menstrual cycles the biphasic temperature curves, the endometria, and the menstrual patterns simulated those that preceded therapy.

The following results indicate that the effects of the hormonal combination were mediated by a functional corpus luteum, since similar treatment during the follicular phase of the normal menstrual cycles did not evoke temperature elevations or secretory (progesteronic) endometrial changes, did not influence the maturing ovarian follicle, and did not change the time of ovulation and luteinization. Three women with normal menstrual

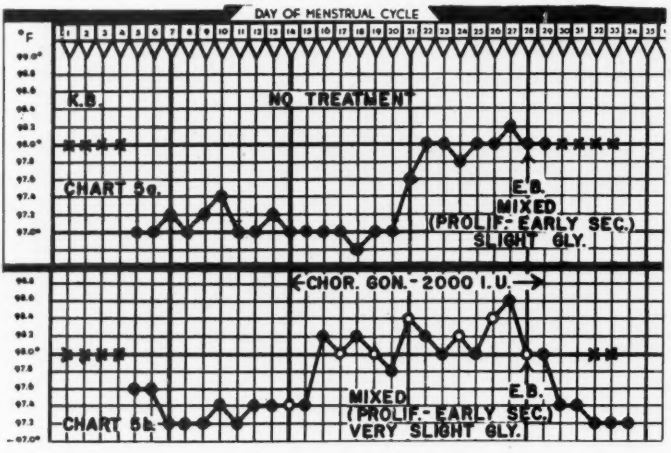


FIGURE 5

cycles received the gonadotropins during the six to ten preovulatory days of the follicular (estrogenic) phase of the cycle. The biphasic temperature curves duplicated those of the controls, and the preovulatory endometria were of the proliferative (estrogenic) type (Figs. 1(f, g); 2(b); 3(d)). The hormones did not modify the low temperature levels and the proliferative endometrium of 1 patient (H.S.) with anovulatory cycles, nor the low temperatures of the 2 castrates.

Regressing corpora lutea were neither revived nor maintained by therapy. Two patients, in whom corpus luteum function had been prolonged by therapy in previous cycles, received daily injections of the effective doses during the four days that preceded the anticipated date of menses, without a change in date or type of flow. A course of therapy, however, that was started just previous to the expected time of regression of the corpus luteum prolonged its normal function (Fig. 3b).

The trauma of endometrial biopsies has been considered to be responsible for a decidual type of endometrial response in some cases. This did not

occur in these patients after repeated biopsies in the proliferative phase (Fig. 1f), nor were the results of effective therapy influenced by repeated biopsies (Figs. 1(e, f, g)).

Antihormone studies were made[6] on serum that was drawn from a patient one week after the completion of 3 consecutive courses of effective combined gonadotropin therapy. The lactogenic hormone, a sheep pituitary extract, elicited antihormone formation as demonstrated by the Leathem method (24). The rat ovarian weights after administration of a crude sheep anterior pituitary extract alone were 61, 41 and 36 mg., in contrast to ovarian weights of 29, 27 and 24 mg., after the injection of the extract and the patient's serum.

Rat ovarian hyperemia pregnancy tests were carried out every third day during the two most prolonged episodes of corpus lutem function (Figs. 2(b); 3(b)). Weak transient positive responses were noted the day before the onset of bleeding in both cycles. The responses were negative before and after those dates.

The effective dosages of the combined therapy did not cause any local or general toxic reactions.[7]

## DISCUSSION

Maintenance of corpus luteum function in this study by the combination of chorionic gonadotropin and prolactin in certain effective dosages was suggestive of a synergistic action by the two gonadotropins, since there was no effect upon the corpus luteum by similar or smaller doses of either hormone alone or by combinations of lesser amounts. The results of this study with 2,000 I.U. of chorionic gonadotropin and 200 units of lactogenic hormone given every third day were comparable to those of Brown and Bradbury (11), who used a special chorionic gonadotropin preparation alone in daily doses of 20,000 I.U. These effects may be explained a) by the possibility of "contamination" of the chorionic gonadotropin with lactogenic hormone (25), b) by Segaloff's recent observation (26) of elevated urinary excretion levels of lactogenic hormone during the administration of the "luteotropic" doses of chorionic gonadotropin, and c) by the synergistic relationship of the luteinizing and luteotropic hormones.

In a report on the luteotropic action of chorionic gonadotropin, Bradbury and his colleagues (19) considered the designation of prolactin as the luteotropic hormone in women, to be premature. This was based on the

[6] The assays were kindly performed for us by Dr. James Leathem, Rutgers University, New Brunswick, N. J.

[7] Since the completion of this study, local urticarial reactions have been noted in a few patients. A mild general reaction was reported in 1 case, but the subsequent injections were uneventful.

inability of these authors to achieve any luteotropic effects in normal cyclic women with daily injections of as much as 1,000 I.U. of lactogenic hormone, and also on the observation that this property of the hormone had been demonstrated only in a species in which there normally occurs a corpus luteum of lactation, namely, the rat. The investigations of Kupperman and Fried (18) and of Hisaw (10) have suggested that the lactogenic hormone by itself does not exert a luteotropic effect in women or monkeys. The finding of a pattern of urinary lactogenic hormone excretion during the secretory phase of the menstrual cycle (20), Everett's previously mentioned experiments in rats (21), and our present study, however, have led us to believe that the lactogenic hormone in combination with the luteinizing hormone has a luteotropic capacity in the human. The response of the human ovary to a purified pituitary luteinizing hormone and to this hormone plus prolactin is at present under investigation by us.

It is generally believed that chorionic gonadotropin in increasing amounts is responsible for the maintenance of the corpus luteum of pregnancy during the first trimester, that is, until the corpus luteum's function of steroid production for the maintenance of pregnancy may be taken over by the placenta (11, 16, 27–29). In this respect it is of interest to note that, as therapy was continued in our patients, the period of corpus luteum activity could not be maintained indefinitely, since a phase was reached during which the onset of bleeding occurred despite continuation of therapy. This necessity for increasingly larger amounts of chorionic gonadotropin for maintenance of the ageing functional corpus luteum has been demonstrated by Browne and Venning (27, 28) and by Brown and Bradbury (11). We also noted that prolongation of corpus luteum function required the initiation of gonadotropin therapy prior to the onset of corpus luteum regression, which occurs at about the tenth to twelfth postovulatory day (27, 28), suggesting that chorionic gonadotropin is responsible for the transformation of the corpus luteum of the menstrual cycle to the corpus luteum of pregnancy. This is supported as well by the reported presence of trophoblastic production of chorionic gonadotropin before the first "missed" period (16, 28, 29). In 5 patients, we have noted sufficient amounts of urinary chorionic gonadotropin on the fourteenth postovulatory day[8] or earlier, to evoke positive responses with rat ovarian hyperemia pregnancy tests (30).

On the basis of the observations presented, the clinical use of a combination of chorionic gonadotropin and prolactin may be indicated in some cases of menstrual dysfunction, sterility, and habitual abortion due to impaired corpus luteum function. Of particular interest is the sterile patient in whom the fertilized ovum fails to implant because of inadequate

[8] Evidenced by waking temperature records.

function or maintenance of the corpus luteum, as evidenced by a short luteal phase and/or by the presence of an imperfect endometrium. This may also include the sterile patient who aborts habitually in the first cycle without knowing it, and the known early aborter in whom the corpus luteum fails in early pregnancy. Studies on patients of these types are now under way.

A syndrome of menorrhagia based on a persistent corpus luteum and associated with irregular shedding of a secretory endometrium has been reported (31). A similar finding was noted in some of our treated patients, in that each episode of persistent corpus luteum function was followed by a menstrual flow of increased duration and amount. In these patients persistent luteal activity was evidenced by the persistence of the secondary temperature elevation throughout the menses, by the premenstrual findings of "full blown" secretory endometria, and by the persistence of urinary pregnanediol premenstrually. The gonadotropin therapy was not responsible directly for the menstrual disturbance but rather indirectly, by its effect on the corpus luteum, since scant menses followed such therapy in the patient (H.S.) with hypomenorrheic, anovulatory cycles.

Of related interest are the findings we have reported in a group of sterile patients in whom the pseudocyesis syndrome developed. Many features of this syndrome were due to persistent ovarian luteinization (32). Recurrent episodes of pseudocyesis frequently followed a symptom or sign that aroused any suspicion of pregnancy. Two sterile patients in the present investigation lent themselves well to a comparative study. Each had the history of sterility and the intense desire for children. During a course of treatment with chorionic gonadotropin and prolactin each considered herself to be pregnant because of the menstrual delay, the prolonged postovulatory temperature elevations, and the positive response to a pregnancy test. The typical pseudocyesis syndrome failed to develop, however, because certain predisposing psychogenic factors were absent, as indicated by psychiatric study.

## SUMMARY

1. A combination of 2,000 I.U. of chorionic gonadotropin and 200 units of lactogenic hormone given every other day during the postovulatory phase of the menstrual cycle prolonged the function of the corpus luteum in regularly menstruating infertile women up to thirteen days, as evidenced by examinations of endometrial tissues, urinary pregnanediol assays and waking temperature charts. Therapy during the preovulatory phase did not effect ovulation. The hormone therapy was very well tolerated.

2. During treatment all postovulatory endometria were normal (secre-

tory, with glycogen) in character in contrast to the mixed (estrogenic-progesteronic) endometria or secretory endometria with glycopenia frequently noted in the control cycles.

3. These effects of the hormonal combination, mediated by a functional corpus luteum, were present only if therapy was started prior to corpus luteum regression, and were demonstrable only during the menstrual cycle in which the gonadotropins were used.

4. There was no effect upon the function of the corpus luteum by similar or smaller doses of either hormone alone or by combinations of lesser amounts.

5. The findings suggest that the lactogenic hormone in synergism with a luteinizing hormone has luteotropic properties in the human.

6. The study offers further evidence for postulating a) a syndrome of menorrhagia due to a persistent functional corpus luteum, b) the transformation of the corpus luteum of the menstrual cycle to the corpus luteum of pregnancy and its maintenance during the first trimester of pregnancy by the chorionic gonadotropin of the trophoblast.

7. Clinical trial of the combination of chorionic gonadotropin and prolactin is suggested for certain cases of menstrual dysfunction, sterility and habitual abortion due to abnormalities of corpus luteum function.

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# THE ACID PHOSPHATASE CONCENTRATION OF THE PROSTATIC EXPRIMATE DURING NORMAL PUBERTY*

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A SIMPLE rapid method of measuring androgenic activity is greatly needed. The determination of the acid phosphatase concentration of the prostatic exprimate seems to supply a practical and reliable means of estimating the androgenic function in children and young adults. Acid phosphatase with an activity optimum near pH 5 is found in a very high concentration in the prostatic secretion of normal young men, the mean value for ages 20 to 40 years being 14,400 units per ml. (1). Between the ages of 40 and 100 years the phosphatase activity of the prostatic exprimate shows a gradual decline to an average value of 3,730 units per ml. for individuals over the age of 80 (1). As demonstrated by Kirk (2) this decline seems to be conditioned, at least partly, by involutionary changes in the prostatic gland.

The existence in older children and in younger adults of a correlation between the androgenic activity and the secretion of acid phosphatase by the prostate has been substantiated particularly through recent extensive studies by From Hansen (3, 4), who showed that patients with clinical signs of hypogonadism have either a low acid phosphatase concentration or none in the prostatic exprimate. He further demonstrated that treatment of such persons with chorionic gonadotropin or testosterone usually is capable of bringing about increased production of enzyme by the gland.

These clinical observations are in good agreement with the earlier findings by Gutman and Gutman (5) in rhesus monkeys. They found that the prostate of infantile rhesus monkeys does not contain acid phosphatase, whereas the gland of mature monkeys does. Gutman and Gutman further proved that it is possible by treatment of preadolescent monkeys with testosterone propionate to induce production of acid phosphatase by the

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prostate, so that following androgen administration the concentration of the enzyme in the gland will be equal to that found in fully grown monkeys.

From the foregoing observations, the behavior of the enzyme as a secondary sex characteristic seems to be established. The physiologic substrate for the enzyme is believed by Lundquist (6) to be phosphoryl choline secreted by the seminal vesicles, but the significance of the function of the enzyme is not known.

Comprehensive studies on the time of appearance and the concentration of the acid phosphatase in the prostatic secretion during normal puberty in humans were not available previous to our recent report (7). Investigations have been reported by From Hansen (4) on 8 normal and 14 imbecile boys between the ages of 13 and 17 years. The mean phosphatase values found in the various age groups were: 13–14 years, 3,600; 14–15 years, 4,660; 15–16 years, 4,700; and 16–17 years, 10,150 units per ml.

Observations have further been made by From Hansen (4) on 5 normal boys, who were followed over a period of from nine to twenty months during the onset of puberty. It was found that the first secretion produced by the prostate contains no phosphatase and that the concentration of the enzyme increases only slowly after it has first made its appearance. This spontaneous slow rise in acid phosphatase concentration during normal puberty is in contrast to the rapid rise in enzyme activity (usually occurring within two to three months) as a result of treatment of hypogonadic individuals with chorionic gonadotropin or testosterone.

The present study was undertaken to determine the normal time of appearance and the concentrations of acid phosphatase in the prostatic exprimate characteristic of the onset and progression of male puberty.

The investigation included 71 boys aged 11 to $16\frac{1}{2}$ years, who were residents of an institution for delinquent children. The boys were first subjected to a clinical examination, and only children who did not show signs of endocrinologic disease were included in the study. The prostatic exprimate was expressed by digital prostatic massage and collected in capillary glass tubes, which were immediately sealed in the pointed flame of a pocket-sized alcohol automatic blast burner. The collections were made in the afternoon in order to minimize the effect of a possible preceding nocturnal seminal emission on the phosphatase values. In all the boys an evaluation of the size of the prostatic gland, and of the degree of development of the external genitals and pubic and axillary hair was made.

The determination of the enzyme activity was performed with minor modifications, as described by From Hansen (3, 4), using disodium phenyl phosphate as the substrate. All analyses were made in duplicate; the results of the duplicate analyses usually agreed within 5 per cent.

## METHOD

Since the method is simple and rapid enough for general clinical use, it is described completely:

### *Method for determination of acid phosphatase activity*

*Reagents:*

1. Citric acid-sodium citrate buffer of pH 4.9, prepared by dissolving 21 Gm. of citric acid and 7.5 Gm. of sodium hydroxide in 1 liter of distilled water. The pH of the prepared solution should be checked electrometrically.
2. Sodium phenyl phosphate, pulverized.
3. Folin and Ciocalteu's (8) reagent. For preparation, see (9). The reagent is kept in the refrigerator and should be discarded if it assumes a greenish tint. It is advisable to check the Folin-Ciocalteu reagent occasionally for its ability in color production. This can be done by preparing phenol standards containing 0.1, 0.2, and 0.5 mg. of phenol and comparing the intensity of color developed after addition of the reagent and sodium carbonate solution. The colors produced in the standards must show proportionality.
4. Sodium carbonate solution, 20 per cent.
5. Phenol standard solution, 0.2 per cent in distilled water. The phenol content of the solution may be checked by iodometric titration of the phenol, according to Holde (10).

*Procedure:*

A 10-cu. mm. portion of prostatic exprimate is added to 5 ml. of a 0.9 per cent sodium chloride solution contained in a glass-stoppered measuring cylinder, and the contents carefully mixed. For measuring the 10-cu. mm. sample a Lang micropipette may conveniently be used. If less than 10 cu. mm. of secretion is available, a correspondingly smaller amount of saline is taken.

A portion of the citric acid-sodium citrate buffer is then heated to 70°C. in order to destroy phosphatase activity caused from growth of fungi, after which the buffer is allowed to cool to 40–43°C. At this stage a phenol standard is prepared by pipetting off 10 ml. of the buffer into a test tube and adding 50 cu. mm. of the 0.2 per cent phenol solution. This standard contains 0.1 milligram of phenol.

To the remaining part of the buffer solution is added 110 mg. of disodium phenyl phosphate powder per each 10 ml. of buffer. After the substrate has dissolved, 10-ml. portions of the still warm solution are pipetted into test tubes. One of these 10-ml. samples serves as a blank.

A 100-cu. mm. portion[1] of the diluted exprimate is pipetted into the 10 ml. of prewarmed buffer-substrate solution contained in the test tube, the contents are mixed by vigorous shaking and the tube placed in a water bath at 37°C. for exactly fifteen minutes. After this period the enzymatic action is stopped by addition of 1 ml. of Folin-Ciocalteu's

[1] We recommend performing duplicate analyses with 50 and with 100 cubic millimeters of the diluted prostatic exprimate rather than with two 100-cubic millimeter samples. This affords further confirmation of the reliability of the individual tests. The same blank must be subtracted from the phenol values observed. With the use of a 50-cubic millimeter sample the amount of phenol liberated by the phosphatase should be multiplied by 40,000 to convert the value into phosphatase units.

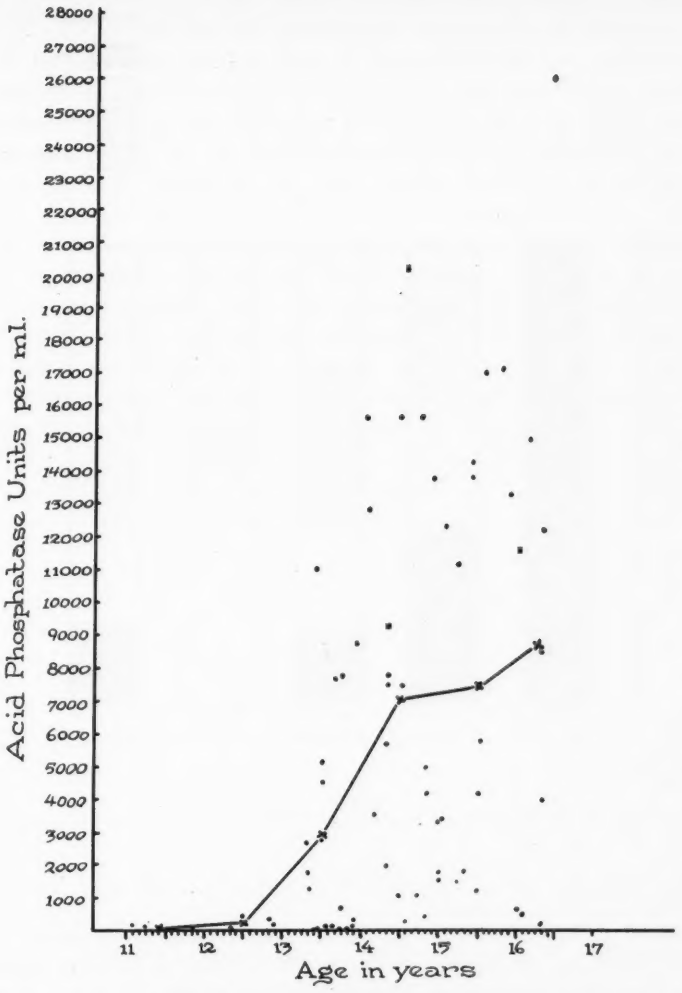


FIG. 1. The acid phosphatase concentration of the prostatic exprimate in 71 normal boys aged 11 to 16½ years.

reagent followed immediately by 4 ml. of 20 per cent sodium carbonate solution. The contents of the tube are mixed and the tube replaced in the water bath for another ten minutes for color development.

The phenol content of the sample is estimated by comparison in a Duboscq colorimeter with the similarly treated phenol standard.

*Calculation:*

From the phenol value found, the value of the blank is subtracted. With the use of

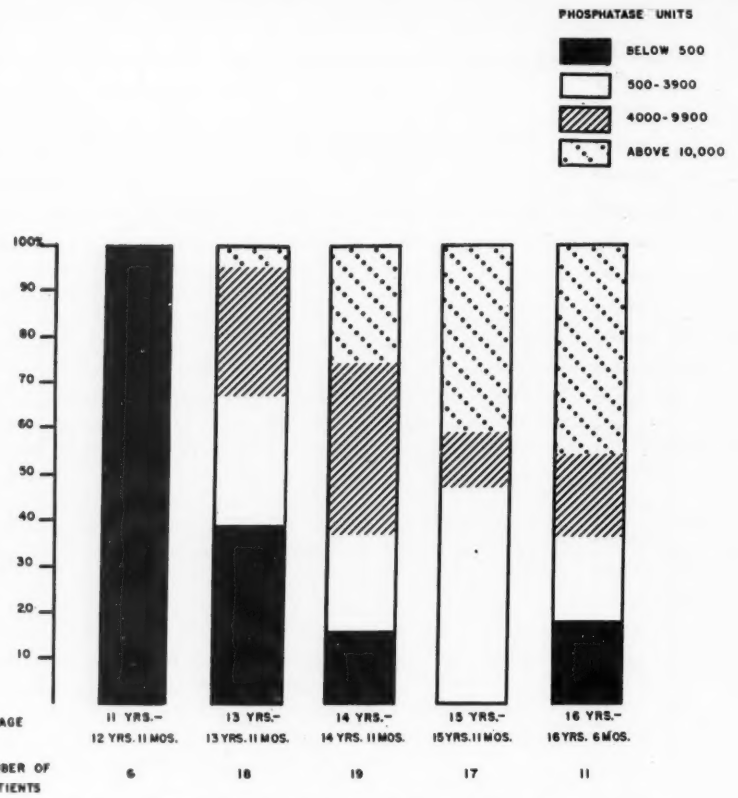


FIG. 2. Acid phosphatase levels in prostatic secretion, by age groups.

high quality disodium phenyl phosphate the phenol value of the blank is low, usually from 0.005 to 0.010 mg. of phenol.

According to the procedure, 1 phosphatase unit represents the enzyme activity capable of liberating 1 milligram of phenol in a one-hour period under the conditions of the test. This phosphatase unit is identical with the Gutman and Gutman unit. Since the quantity of exprimate contained in the final reaction is 0.0002 ml. and the reaction period used is fifteen minutes, the milligrams of phenol liberated in the test must be multiplied by 20,000 to convert the figure into phosphatase units.

## RESULTS

The results of the phosphatase determinations are shown in Figure 1. It will be seen that the enzyme starts to make its appearance between the ages of 11 and 13 years and that after the age of 13 the values tend to increase sharply. The figure further shows that there is a considerable scattering of the values in the 13 to 16½-year age group. This is not unex-

pected in view of the normal variation in age for the onset of puberty. It will be noted that several of the boys between 14 and 16½ years already had reached the adult phosphatase level. A calculation of the mean phosphatase values shows the following phosphatase concentrations for the various age groups: 11–12 years, 50; 12–13 years, 200; 13–14 years, 2,880; 14–15 years, 7,020; 15–16 years, 7,380; 16–16½ years, 8,700 units per ml.

Figure 2 demonstrates the distribution of acid phosphatase levels of the prostatic secretion by age groups. It will be seen that there is a rapid decrease in the number of boys with acid phosphatase levels of below 500 units after the age of 12. By age 16, approximately 50 per cent of the group have values of 10,000 units or above.

It is of interest to compare the acid phosphatase levels of this group of adolescent boys with the mean values observed in a previous study (1) of 200 adults aged 20 to 100 years. Figure 3 shows that at the age of 16 to 16½ years the mean phosphatase value is still definitely lower than the mean value for subjects aged 20 to 29.

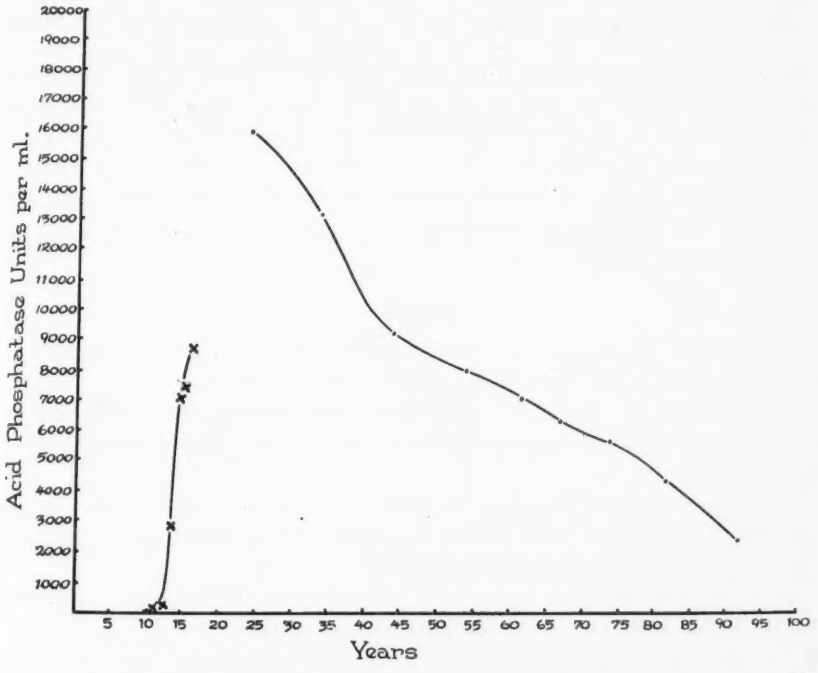


FIG. 3. The mean acid phosphatase concentration of the prostatic exprimate in 71 normal boys and 200 adults.

Table 1 illustrates the correlation between clinical signs of sexual development and observed phosphatase values. As seen from the table, a good correlation was found between the size of the external genitals, size

TABLE 1. SEX CHARACTERISTICS CORRELATED WITH MEAN ACID PHOSPHATASE LEVELS

| SIZE OF GENITALIA | | |
|---|---|---|
| SMALL | MODERATE | ADULT |
| 1220 | 2081 | 8300 |

| SIZE OF PROSTATE | | | |
|---|---|---|---|
| NOT PALPABLE | SMALL | MODERATE | ADULT |
| 895 | 1653 | 6354 | 10418 |

| PUBIC HAIR | | | |
|---|---|---|---|
| ABSENT | SLIGHT | MODERATE | ABUNDANT |
| 188 | 1550 | 6332 | 10476 |

| AXILLARY HAIR | | | |
|---|---|---|---|
| ABSENT | SLIGHT | MODERATE | ABUNDANT |
| 1994 | 6820 | 9892 | 14345 |

of the prostate, amount of pubic hair, amount of axillary hair and mean phosphatase value. In a few cases, however, the correlation was not close. Thus of 12 boys between the ages of 14 and $16\frac{1}{2}$ years, whose phosphatase levels were below 2,000 units, 5 were considered to be sexually mature. In these 5 boys the observed values were 410, 1,060, 1,480, 1,760, and 1,760 units. In only 1 sexually immature individual was a high phosphatase value (7,600) observed.

## SUMMARY

1. Acid phosphatase levels of the prostatic exprimate were determined in 71 normal boys between the ages of 11 and $16\frac{1}{2}$ years.

2. There was an increase in the mean acid phosphatase level from 50 phosphatase units per ml. of prostatic fluid at age 11, to 8,700 units at age 16 to $16\frac{1}{2}$.

3. A wide variation in acid phosphatase levels was found between the ages of 13 and $16\frac{1}{2}$, a fact which corresponds with the normal variation in the age of boys at the onset of puberty.

4. A good correlation was noted between the clinical signs of sexual development and the mean acid phosphatase values.

5. This method should be of use as a simple laboratory means of confirming clinical diagnoses of male hypogonadism in children and young adults.

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# EFFECT OF CORTISONE ON TOLERANCE TO ASCORBIC ACID AND LEVEL OF BLOOD CHOLESTEROL IN MAN*†

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## INTRODUCTION

EXPERIMENTAL studies with animals (1, 2, 3) as well as clinical studies have been reported, which suggest a relationship between ascorbic acid metabolism and adrenocortical hormones. The therapeutic use of ascorbic acid in large doses and of ascorbic acid in conjunction with adrenocortical hormone administration has been proposed in the treatment of collagenous diseases (4, 5, 6, 7). Our own experimental observations and those of others on the diminution of ascorbic acid excretion following adrenocortical hormone administration provoked our curiosity as to what might occur in the various patients during cortisone therapy. Selye (8) postulates that a major factor in the pathogenesis of certain diseases, now treated with cortisone, is failure of the particular organism to adapt to stress. It has been shown repeatedly that during stress adrenal ascorbic acid and cholesterol decrease (Sayers, 9). In a previous report (10), we have suggested that the increase in serum cholesterol following ascorbic acid administration may represent adrenal gland release of cholesterol concomitant with storage of ascorbic acid in the gland, although it is questionable whether the hypercholesterolemia is due entirely to adrenal gland response.

A comparison of the changes in the ability to handle ascorbic acid and in the patterns of cholesterol response to ascorbic acid in patients before

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and during treatment with cortisone may well serve as a means to shed more light on the question of the role of ascorbic acid in adrenocortical hormone formation and function; hence the importance of this work.

## METHODS

Observations were made on a group of 30 patients. This group included patients with rheumatoid arthritis, chronic ulcerative colitis, psoriasis, scleroderma, disseminated lupus erythematosus, leukemia and drug sensitivity.

Before cortisone treatment all patients were arranged for a study of tolerance and of blood chemistry changes following ascorbic acid administration. A blood sample was drawn and urine was collected with the patient in a fasting state, following which 300 mg. of ascorbic acid was administrated intravenously. Blood and urine were collected hourly thereafter for three hours. Ascorbic acid was determined on plasma and cells, using

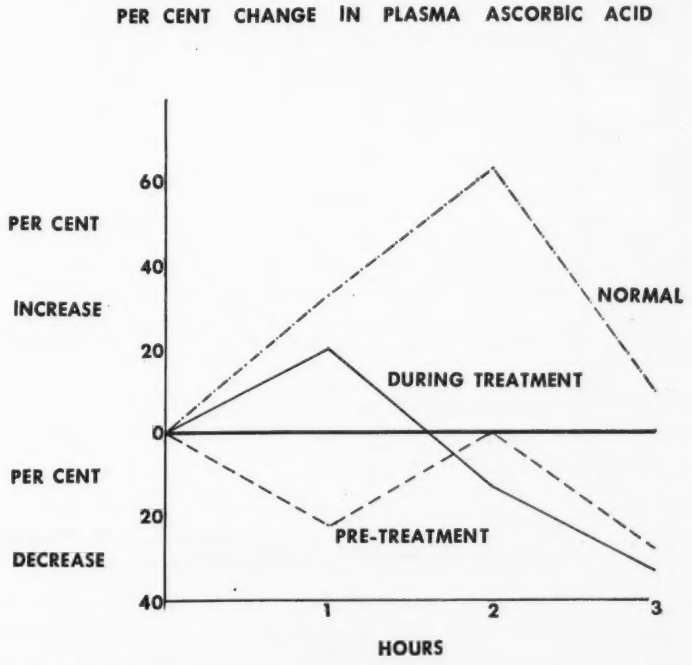


FIG. 1. Showing the mean percentage change of the plasma ascorbic acid of 20 patients before and during cortisone therapy, following the intravenous administration of 300 mg. of ascorbic acid Also shown is the mean percentage change of plasma ascorbic acid in 10 normal subjects, following administration of 300 mg. of ascorbic acid intravenously. P = <.01 (pre-treated and treated periods compared at first hour.)

the method of Mindlin and Butler (11). Urinary ascorbic acid was determined by titration with standard 2, 6 dichloroindophenol. Serum cholesterol concentration was determined by the method of Bloor *et al.* (12), blood chlorides by the well known procedure of silver nitrate precipitation and titration against standard potassium thiocyanide, blood sodium by the Weinback modification of the Butler and Tuthill method (13), and potassium by the method of Shohl and Bennett (14).

Following one to ten days of continuous cortisone therapy, beginning the dose at 300 mg. daily and reducing it to 50–75 mg. daily, the ascorbic acid tolerance and blood chemistry changes were again studied under the identical conditions described for the pre-cortisone period. In some in-

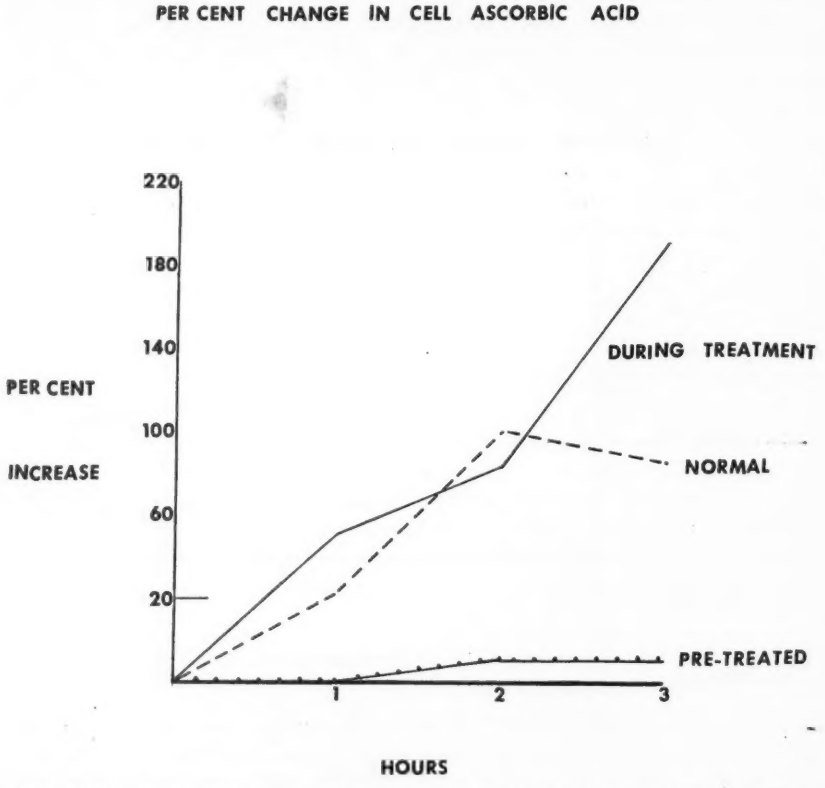


FIG. 2. Showing the mean percentage change in the blood cell ascorbic acid in 20 patients before and during cortisone therapy, following administration of 300 mg. of ascorbic acid intravenously; and the results in 10 normal subjects, following the same ascorbic acid dosage.

P = < .01 (pre-treated and treated periods compared at first hour.)

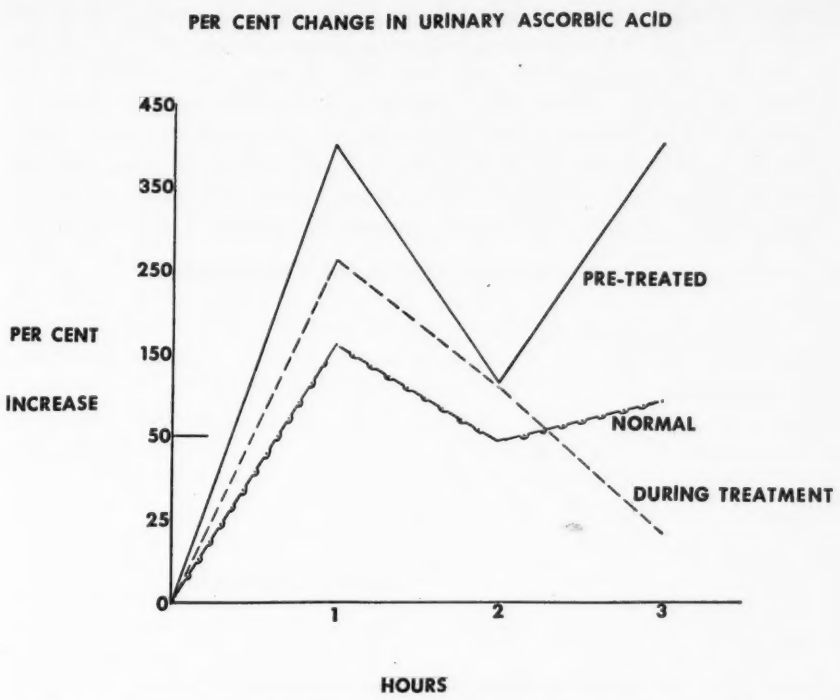


FIG. 3. Showing the mean percentage change in the urinary excretion of ascorbic acid in 20 patients before and during cortisone therapy, following administration of 300 mg. of ascorbic acid intravenously; and the results in 10 normal subjects following the same ascorbic acid dosage.

P = <.01 (pre-treated and treated periods compared at first hour.)

stances, a second test was made during the period in which cortisone was administered.

## RESULTS

Figure 1 shows the percentage changes in plasma ascorbic acid following its administration to normal subjects, to patients before cortisone treatment and to the same patients during cortisone treatment. Note the great increase in the level of plasma ascorbic acid in the normal subjects in the first and second hours and the return almost to the initial level by the third hour. On the other hand, in the patients before cortisone treatment, the plasma ascorbic acid showed a decrease in the first hour, returned to the initial level in the second hour and decreased again in the third hour. During cortisone treatment, the ascorbic acid tolerance curve changed toward that of normal subjects as shown by the 20 per cent increase in

plasma ascorbic acid in the first hour, although in the second hour the value decreased 18 per cent and in the third hour, 32 per cent. The change in the tolerance to ascorbic acid and the influence of cortisone on the permeability of the blood cells to ascorbic acid can be seen in Figure 2. Note the lack of increase in cell ascorbic acid in the first hour after ascorbic acid was administered to patients prior to cortisone treatment, and the very slight increase in the second and third hours; whereas the same patients during cortisone treatment showed tremendous increases in cell

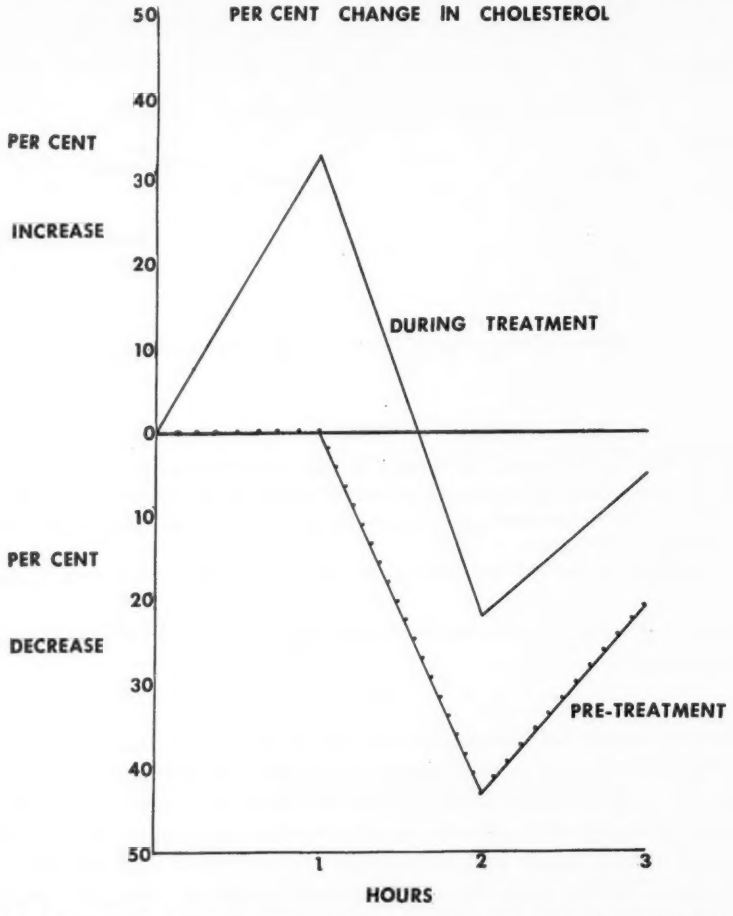


FIG. 4. Showing the mean percentage change in the total serum cholesterol in 20 patients before and during cortisone therapy, following administration of ascorbic acid intravenously.

P = <.01 (pre-treated and treated periods compared at first hour.)

ascorbic acid in the first, second and third hours. It is significant that the percentage increase was greater than that of normal subjects at all time intervals. The increase in tolerance to ascorbic acid during cortisone treatment is again shown in Figure 3. During cortisone treatment the urinary output of ascorbic acid was far less than in the same patients before cortisone was administered. In the first hour the pre-treated cortisone patients excreted approximately 150 per cent more ascorbic acid than did the same patients during cortisone treatment and approximately 250 per cent more than did the normal subjects. Note that in the second hour, individuals in all categories showed a decreased output, but in the third hour the patients undergoing cortisone treatment showed an excretion which was decreased far below that of normal subjects. The changes in the level of serum cholesterol following ascorbic acid administration in the pre-treated and treated periods can be seen in Figure 4. It is interesting that prior to cortisone treatment the cholesterol did not change in the first hour following ascorbic acid administration, but decreased as much as 42 and 20 per cent in the second and third hours, respectively; whereas the same patients during cortisone therapy showed an increase of approximately 33 per cent in serum cholesterol in the first hour, followed by decreases, of 21 and 5 per cent in the second and third hours, respectively. The statistical significance of the data presented in Figures 1, 2, 3 and 4 is expressed in the legends for the figures.

## DISCUSSION

The results of these studies are in agreement with experimental observations made in these laboratories that adrenal steroids reduce the urinary excretion of ascorbic acid and cause higher blood levels to be built up, particularly in the blood cells. The increase in cell content following the administration of ascorbic acid during cortisone therapy is outstanding, and favors the view that cortisone increases cell permeability. Increase in the kidney threshold to ascorbic acid may explain the increase of ascorbic acid in the plasma concomitant with reduced excretion in the urine.

The meaning of the changes in the level of blood cholesterol before and during cortisone treatment is not clearly defined at this time. We postulate that the normal functioning adrenal gland stores ascorbic acid upon administration to the organism; this storage may be increased by exogenous hormones. There is evidence to show that as the adrenal ascorbic acid increases, the adrenal cholesterol decreases (1). We believe, therefore, that the elevated serum cholesterol may represent, in part, cholesterol which has been released by the adrenal gland. Conclusion on this point will have to await attempts to calculate the actual amount of cholesterol that may be leaving the gland following ascorbic acid administration.

In this connection, however, it is significant that the Addisonian patient and adrenalectomized animals show no significant increase in cholesterol following ascorbic acid administration. Nor does the administration of adrenal steroids, in the absence of the adrenal glands, result in an increased level of serum cholesterol (15). If our thesis is correct, that the increase in serum cholesterol concentration is concomitant with increased storage of ascorbic acid in the adrenal gland, it may be tenable that patients during treatment with cortisone are storing administered ascorbic acid in the adrenals in contradistinction to their response during the pre-treated periods. We have shown (1), as have Schaffenburg, Masson and Corcoran (7), that cortisone increases adrenal ascorbic acid storage in experimental animals. The role of the endogenous adrenocortical hormone in ascorbic acid and cholesterol metabolism, however, is not clear cut. It is well known that, following ACTH administration and during stress, the cholesterol and ascorbic acid content of the adrenal decreases. During such conditions, it is thought that adrenocortical activity is increased. We have called attention (3) to experiments in which the fall in adrenal ascorbic acid and cholesterol could be blocked by the administration of adrenocortical hormones. Also we have pointed out the increased deposition of ascorbic acid in the adrenal glands of animals receiving ascorbic acid and adrenocortical extract, compared with the deposition in animals receiving only ascorbic acid. It is understandable, therefore, that patients under cortisone therapy should excrete less ascorbic acid than before. We are not clear, however, as to the role ascorbic acid may be playing in the formation of adrenocortical hormones or its influence on the functional state of the adrenal gland. Schaffenburg, Masson and Corcoran (6) have mentioned that the adrenal gland in scurvy, in experimental arthritis and in Addison's disease is depleted of its ascorbic acid, and is therefore deficient in glucosteroid. If this view is correct, ascorbic acid may play an outstanding part in the formation of the adrenocortical hormone and in the state of function of the adrenal gland.

## SUMMARY AND CONCLUSION

1. Experiments are described showing the influence of cortisone on ascorbic acid metabolism in patients.

2. When ascorbic acid is given intravenously during cortisone therapy, there is a greater increase in the level of blood cell and plasma ascorbic acid and the levels remain increased longer than in the same patients prior to cortisone therapy. The increase in the blood cell value is particularly noteworthy.

3. When ascorbic acid is given intravenously during cortisone therapy, there is decreased urinary excretion of ascorbic acid in spite of increased

plasma levels, suggesting that cortisone causes an increase in the kidney threshold to ascorbic acid.

4. The pattern of serum cholesterol response to ascorbic acid was reversed during cortisone therapy in the patients studied. Prior to cortisone therapy the level of serum cholesterol was unchanged in the first hour following intravenous administration of ascorbic acid, whereas in the same patients during cortisone therapy, the level of serum cholesterol was elevated in the first hour. The possible relationship of this elevation to ascorbic acid storage in the adrenal gland is discussed.

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# STUDIES ON THE MECHANISM OF THE SPERMATIC RELEASE OF THE MALE NORTH AMERICAN FROG (*RANA PIPIENS*)

## REACTION TO VARIOUS HORMONES*

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THE response of the male frog (*Rana pipiens*) to gonadotropins is by now well known (1, 2, 3). This highly useful reaction forms the basis of the widely used male frog diagnostic test for pregnancy. It is less well known that these amphibia will also react with great constancy to adrenalin (4). Except for the report of Greenblatt (5) and a few diverse endocrinologic observations made as control studies in the course of pregnancy diagnostic work, very little is known about the male frog in regard to the pathways and mechanisms by which an injected substance is absorbed, disseminated and evokes the release of motile sperm. The relationship of the adrenalin reaction to the gonadotropic response is also unknown. The suggestion made by Greenblatt *et al.* that adrenalin and gonadotropin act through the pituitary cannot be confirmed by us (6).

Several excellent anatomic studies are available which provide a relatively clear picture of the genito-urinary and endocrine systems of the male frog (7, 8). Even in this better established field of morphology, however, doubt still exists as to whether a functioning adrenal gland is present in the *Rana pipiens* (9). Nests of apparent adrenal cortical cells but no medullary cells are found dispersed along the ventral surface of the kidneys, but attempts at elucidating their function have proven inconclusive. The widespread use of male frogs is based, then, largely on a few scattered observations.

It is the purpose of this communication to present the reactivity of these amphibia to commonly used endocrine preparations, in an attempt to clarify the mechanism of spermatic ejaculation in these lower animals.

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* We are indebted to Ciba Pharmaceutical Products, Inc., Summit, New Jersey, for the support of this work.

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## MATERIAL AND METHODS

All of the male frogs (*Rana pipiens*) used in this experiment were adult, each weighing approximately 20 to 35 grams. These animals were obtained from commercial collectors who gathered them from their native habitat in the spring of the year and kept them for yearly distribution in enforced hibernation in wire cages sunk to the bottom of native ponds. During the months of March and April when the new supply is being gathered, it is not uncommon to have occasional males emit sperm after nonspecific stress stimuli such as handling or trauma. This difficulty is readily overcome by keeping the animals in tanks in the laboratory for a few days prior to their use, during which time basal conditions appear to be re-established. All experimental procedures were performed on frogs either in pairs or on 3 or 4 animals in parallel. Usual laboratory animals are bred under standard conditions; their age, adequacy of nutrition, general state of health and vigor are fairly well established and standardized at the time of their use. Obviously none of these facts can be well known about frogs and only size can be used as a criterion for suitability. Minor variations, therefore, in sensitivity and reactivity exist between various animals that can be controlled only by the use of numbers of frogs in parallel.

The drugs and preparations administered to these amphibia were given subcutaneously into the dorsal lymph sac, unless otherwise indicated. As far as possible, the hormones were administered in solution but occasionally finely divided suspensions had to be employed. Cloacal smears were made, as with the ordinary pregnancy test, at half-hourly intervals for at least two to three hours and occasionally for longer periods of time when necessary. In general, the time required for the development of a reaction varied between twenty and forty minutes.

Irrespective of whether a positive or negative response was elicited, the frogs were used only once.

## EXPERIMENTAL DATA

The various preparations administered to the frogs and the responses elicited are summarized in Table 1.

## DISCUSSION

Study of the preceding chart indicates that the variety of substances used can be divided on the basis of the frog response into three categories: 1) those substances that invariably evoke a reaction and to which the male frog seems highly sensitive, 2) those substances which inconstantly produce reactions, and 3) the remainder, which are entirely negative. The intermediate substances that occasionally produce gametokinesis require

TABLE 1

| Preparation | Dose | Reaction |
|---|---|---|
| *Pituitary* | | |
| Pituitrin (Parke-Davis) | 10 I.U. | 0 |
| Pitressin (Parke-Davis) | 20 pressor U. | 0 |
| Polyansin (Armour) | 1–1.5 cc. | 0 |
| FSH (McShan)* | 10 mg. | ++++ |
| Menopausal urine (alc. ppted.) | 100 cc. | ++ |
| Postmenopausal urine (whole) | up to 5 cc. | 0 |
| Postmenopausal urine (acet. ppted.) | 40 cc. | 0 |
| Postmenopausal urine (alc. ppted.) | 100 cc. | 0 |
| Thyrotropin (Armour) | 1 Rowland-Parkes U. | 0 |
| Gonadophysin (Searle) | down to 1 R.U. | ++++ |
| *Placenta* | | |
| Antuitrin S. (Parke-Davis) | down to 40 U. or 0.625 R.U. | ++++ |
| Pregnancy urine (whole) | 5 cc. | ++++ |
| Pregnancy urine (20 cc. ppted. with alc.) | 5 cc. | ++++ |
| *Adrenal* | | |
| Corticotropin | 40–60 mg. daily | 0 |
| Percorten (desoxycorticosterone) (Ciba) | 5 mg.—7 days | 0 |
| Aqueous adrenal extract (Upjohn) | 1–2 cc. (5–10 dog U.) | ± |
| Lipo-adrenal extract (Upjohn) | 0.5 mg. | 0 |
| Cortisone (Merck) | 2.5–10 mg. | 0 |
| Cortisone acetate (Merck) | 1–10 mg. | 0 |
| Dehydroisoandrosterone (Ciba) | 0.1 mg. | 0 |
| | 0.2 mg. | 0 |
| | 0.4 mg. | ± |
| | 0.5 mg. | ± |
| | 1 mg. | ± |
| | 2 mg. | 0 |
| 17-Ketosteroids (urinary extract) | 5.35 mg. | ± |
| Adrenalin (natural and synthetic) | 0.001 mg. | ++++ |
| *Ovary* | | |
| Estrogen | | |
| Benovocylin (Ciba) | 35 mg. for 6 days | 0 |
| Sistomen (Ciba) | 25–50 mg. | 0 |
| Stilbestrol | 2 mg. | 0 |
| Emmenin (Ayerst, McKenna & Harrison) | 45 dog oral U. | 0 |
| Pregnancy urine extract | 20 c.m.u. | 0 |
| | 30 c.m.u. | 0 |
| | 40 c.m.u. | 0 |
| Progesterone | | |
| Lutocylin (Ciba) | 2 Gm. daily for 3 days | 0 |

* Obtained through the courtesy of Dr. W. H. McShan, University of Wisconsin.

TABLE 1 (continued)

| Preparation | Dose | Reaction |
|---|---|---|
| *Testes* | | |
| Perandren (Ciba) | 5 mg. for 5 days | 0 |
| Oreton (Schering) | 25 mg. | 0 |
| Androstine B (lipid) (Ciba) | 10–20 mg. | 0 |
| Androstine A (aq.) (Ciba) | | ± |
| *Thyroid* | | |
| Desiccated thyroid | 0.5 Gm. for 3 days | 0 |
| Thyroxine | 0.5 cc. (1–1,000 saline) | 0 |
| *Pancreas* | | |
| Insulin | up to 20 U. reg. | 0 |

large doses and the spermatic response is frequently weak and difficult to reproduce. The significance of these reactions is not clear.

In the first category are found the gonadotropins and epinephrine.

The effectiveness of the chorionic gonadotropins of both animal and human origin in evoking gametokinesis is well known. The minimal dose required to cause spermatic emission is approximately equivalent to the amount required to produce macroscopic corpus luteum formation in an immature white rat. The relation of the chorionic frog unit to the rat unit has remained constant over a great number both of parallel titrations of many pregnancy urine samples and of various manufacturers' lots of hormone.

Pituitary gonadotropin likewise causes a reaction in male frogs. In fact, it has been pointed out earlier that the male frog appears much more sensitive to this hormone than does the immature white rat.

Using pituitary gonadotropins of sheep origin (Gonadophysin, Searle), the frogs reacted to approximately 1/16 the amount required to produce a corpus luteum reaction in rats. With this pituitary gonadotropin, the end point in the frog closely parallels the FSH (follicle-stimulating hormone) end point in the rat. The possibility that the frog might indeed be responding to the FSH was proven by the administration of a purified preparation of this factor obtain from Dr. McShan.[1] Greenblatt *et al.*, who independently used FSH from the same source, namely Dr. McShan, arrived at the same conclusion (5). It is still possible, however, that traces of LH (luteinizing hormone) may still be present in this fraction, and it is therefore possible that the gametokinesis may indeed require LH to potentiate the FSH response. The reaction of the frog to concentrations of menopausal urine fails to shed any light on the question of which pituitary factor is the important one, since menopausal urine is known to contain

[1] Kindly supplied by Prof. W. H. McShan of the University of Wisconsin.

not only FSH but also traces of LH. Until total separation of the FSH and LH fractions is possible chemically, or these fractions can be synthesized, this question must remain unanswered. As will be discussed in greater detail at a later date, hypophysectomy failed to block the reaction to the gonadotropins of either pituitary or chorionic origin.

Epinephrine is another hormone to which the frog appears quite sensitive. The great variety of closely related adrenergic drugs that have been assayed on these amphibia failed to match the effectiveness of epinephrine (4). Both the naturally derived and the synthetic adrenalin were equally effective in amounts down to as little as .001 mg. Obviously the most intriguing aspect of this adrenalin response, apart from its possible practical assay value, is its relationship to the gonadotropic response. Hypophysectomy fails to block the response to epinephrine. This observation precludes the obvious assumption that epinephrine evokes its reaction through the pituitary release of gonadotropic substances. Moreover, there are several distinct differences between the frog response to adrenalin and to the gonadotropins. One of the most significant is that the adrenalin reaction is seasonal. In the months of June, July and August this substance fails to evoke a response. This refractory period, which appears to coincide with the post-mating season of these amphibia, is not shared by the gonadotropins. All attempts to overcome this non-reactive phase with substitution therapy using anterior pituitary extracts, gonadotropins, ovarian hormones and thyroid have failed. Whether epinephrine itself is the ultimate substance that reacts to produce the gametokinesis or whether its effect is mediated through some other hormonal or neural mechanism is not clear at present.

The great variety of other hormones administered to these animals fall into the remaining categories of substances that either entirely fail to evoke a response or do not consistently produce one. Among the non-reacting substances one finds notably the ovarian hormones, both the estrogen and progesterone fractions. Their failure to have any effect on the male frogs adds considerably to the specificity of the diagnostic pregnancy test.

The testicular preparations prove to be of somewhat greater interest. The commercial lipoidal testosterone preparations all failed to produce reactions. However, one aqueous extract of bull testis produced for experimental purposes, Androstine B (Ciba), occasionally caused a positive reaction. There was considerable variability in the effectiveness of this extract and several of the production lots were completely without gametokinetic effect. Since it was not clear precisely what this aqueous extract contained, no basis for this response could be established. Pure histamine preparations were without effect on these amphibia, and it was

thus evident that the histamine content of the extract could have no relation to the causation of the positive reaction.

Perhaps the most interesting responses were obtained with the various adrenal cortical substances. These compounds fall into the category of occasional reactors. To begin with, it should be recalled that adrenocorticotropic hormone failed to produce any reaction in these amphibia. The failure of corticotropic hormone to evoke gametokinesis through its direct stimulatory effect on the adrenal cortices strongly argues against the hypothesis that the adrenalin effect is mediated through the adrenal glands. Certainly it would seem that the stimulatory action on the adrenal cortex of its tropic hormone should be more powerful and more direct than the mild stimulatory effect on the adrenal cortex produced by adrenalin. Notwithstanding the absence of a response to the adrenotropic hormone, certain of the adrenal cortical steroids produced inconstant reactions. Upjohn's lipid and aqueous adrenal extracts, dehydroisoandrosterone, and the mixed alpha and beta 17-ketosteroids obtained from the urine of a normal person were occasionally productive of a positive reaction. Desoxycorticosterone acetate and desoxycorticosterone, as well, as cortisone and cortisone acetate, all gave completely negative findings. The interpretation of these results with the foregoing compounds is most difficult, especially since the reactions were not consistent. Too many positive responses were elicited, however, for them to be dismissed as merely nonspecific errors. It is only possible to hypothesize that the effect of these active steroids was not a powerful one, and that only the most sensitive reactive frogs responded. Increasing the size of the dose, however, even to toxic levels, failed to overcome this theoretic insensitivity of some of the animals. The failure of the adrenotropic hormones to produce a reaction appears to be paradoxic in the presence of the positive reactions to adrenal extracts and steroids. This disparity might be explained on the basis that these reactive steroids are either not produced by the frog or are not produced in sufficient amount to evoke a response. These speculations bear on the question of the existence of a functioning adrenal gland in the frog. Although adrenal cortical cells are apparent within the kidney substance, no tissue resembling medulla has ever been observed. Neither is it clear as to whether the "cortical-like" cells have any function. Most, if not all, of the epinephrine release produced in frogs has been shown to be produced in the skin. At the present time, therefore, this entire problem of adrenalin response and adrenal steroid activity must remain unanswered.

## SUMMARY

A survey of the reactivity of male frogs to various endocrines is reported.

Only the gonadotropins and epinephrine constantly produced reactions. Parallel titrations of chorionic and pituitary gonadotropins on rats and frogs produced certain differences, suggesting that with the pituitary-derived substances the frog may indeed be responding to the FSH factor. Although the gametokinetic effect of epinephrine has been shown to be independent of the pituitary gland, additional conclusions must await further work. The effect on frogs of gonadal and adrenal hormones is discussed.

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# Letter to the Editor

## ACTH THERAPY IN 2 CASES OF RHEUMATOID ARTHRITIS AND PSORIASIS COEXISTING WITH DIABETES MELLITUS

To the Editor:

Diabetes mellitus and rheumatoid arthritis seldom coexist (1). Diabetes is a relative contraindication to cortisone or ACTH therapy, since these drugs further decrease carbohydrate tolerance, as shown in animal experiments reported by several workers.

Conn, Louis and Wheeler (2) noted a temporary diabetic state in 3 normal adults given rather large doses of ACTH. This diabetogenic effect is more evident when the adrenal glands and/or the pancreas are diseased, as shown by Sprague *et al.* (3) in cases of coexisting Addison's disease and diabetes, by Forsham *et al.* (4) in cases of Addison's disease with or without associated diabetes, and by Perera *et al.* (5) in a case of hypertension and diabetes. Related to this fact is the observation of Sprague and associates (6) that 2 of 4 patients whose carbohydrate tolerance was decreased on cortisone therapy, had diabetes in their families.

Boland and Headley (7) reported 2 cases of latent diabetes mellitus (as shown by glucose tolerance tests) treated continuously by cortisone for ninety-eight and seventy-nine days, respectively. There was no change in the results of the carbohydrate tolerance tests and no diabetic symptoms in the first case. There was an irregular glycosuria and more pronounced changes in the glucose tolerance curve in the second case. Neither insulin nor dietary restriction was necessary in either case. Two other cases showed significant decreases in carbohydrate tolerance during cortisone therapy. The authors also reported another patient whose daily insulin requirement was increased fivefold during cortisone therapy.

Brown *et al.* (9) concluded that the therapeutic effects of ACTH or of cortisone on rheumatoid arthritis associated with diabetes bear no apparent relation to carbohydrate changes, it being immaterial whether or not the aggravation of diabetic symptoms is controlled by increased amounts of insulin.

The association of diabetes mellitus, rheumatoid arthritis and psoriasis is even rarer; and, because large doses of ACTH are necessary for psoriasis, it offers a challenge to ACTH or cortisone therapy. The purpose of this communication is to report 2 such patients treated continuously by ACTH for thirty-four and forty-five days, respectively.

CASE REPORTS

**Case 1. J.C.F.** (#2133, Catedrá de Patología Médica, Dr. Iglesias Betancourt), a white, 51-year-old man, had had rheumatoid spondylitis and peripheral arthritis for five years. His right ankle had been so painful and swollen that he had been unable to walk for three months. Psoriasis had been present for two years, appearing at both elbows and progressively involving most of his body; it was most marked on the dorsal aspects of the elbows and knees. His condition had proved resistant to sodium salicylate, gold salts and pregnenolone. For the previous two years he had been treated for diabetes mellitus by means of diet and 40 units of protamine zinc insulin daily. Ten years before, he had had a syphilitic lesion treated by neosalvarsan, bismuth and penicillin.

On physical examination the patient was found to be moderately obese. His height was 1.7 M. and his weight, 83 Kg. The blood pressure was normal. He could walk with great difficulty, and only when using a cane. The left metacarpophalangeal and right tibiotarsal joints were most painful and swollen. Some psoriatic lesions were over 20 cm. in diameter. The rest of the examination showed nothing abnormal.

The blood count was normal and the sedimentation rate was 36 mm. (Wintrobe). Serologic tests for syphilis, and spinal puncture, gave negative results. Values for blood proteins and NPN were normal; blood cholesterol, 312 mg.; and fasting blood sugar, 153 mg. per 100 cc. Urinalysis showed a moderate glycosuria. Thorn's test (Randolph technique) showed 31.5 eosinophils before the administration of 10 mg. of ACTH, and none four hours afterward.

The patient was given the regular hospital diet (containing around 225 Gm. of carbohydrate), 20 units of protamine zinc insulin and 40 mg. of ACTH daily in four divided doses, for a period of ten days. Then ACTH was increased to 80 mg. daily for seventeen more days. Two weeks after beginning ACTH, arthritic symptoms had subsided and the patient was able to walk freely. The sedimentation rate being 16 mm. after four weeks of therapy, ACTH was reduced to 20 mg. daily for a week. (Total dose, 1.98 Gm. in thirty-four days. Average daily dose, 58 mg.) Fasting blood sugar increased to 181 mg., 153 mg., 181 mg. and 194 mg. during therapy, with no change in the daily dose of insulin. The patient was sent to the Out-Patient Department with complete clinical and biologic remission as far as his arthritis was concerned, but his psoriasis was only slightly benefited.

**Case 2. G.G.H.** (private patient), was a 41-year-old white man, whose father and mother were diabetics. His case had been diagnosed as diabetes mellitus when he was 19 years old and he had been treated accordingly since that time. Since he was 29 he had been suffering from psoriasis. When he was 34, peripheral rheumatoid arthritis developed.

On physical examination he was found to be underweight (height, 1.72 M. and weight, 52 Kg.). His temperature was 101° F. (38.5° C.), and his pulse rate, 110. All joints of his four limbs were swollen and painful (Fig. 1), movements being very limited and the patient being unable to stand. Psoriatic lesions were scattered all over his body.

His red blood cell count was 3.45 million and his white count, 14,250. The sedimentation rate (Westergreen) was 52 mm. in one hour and 108 mm. in two hours. The value for blood cholesterol was 120 mg.; fasting blood sugar, 285 mg.; serum sodium, 320 mg.; and potassium, 14.8 mg. per 100 cc. Urinalysis: albumin, 0.5 Gm.; ketonic bodies, 2 plus; glycosuria, 3 plus; RBC, 1 plus; and hyaline casts, 1 plus. An electrocardiogram and fluoroscopy of the chest gave normal findings. Thorn's test with ACTH gave positive results.

The patient was given the regular hospital diet and a 2:1 mixture of 40 units of insulin

FIG. 1. Showing condition of the joints in Case 2.

and 20 units of protamine zinc insulin daily. A few days after the diabetes was well controlled, ACTH was started in a dose of 5 mg. four times daily, slowly increasing to 120 mg. daily in a 2-week interval. This large dose was administered for two weeks. Blood sugar increased and there was glycosuria, so the dose of I and PZI was doubled.

The patient showed complete clinical remission of his arthritis. He was able to move around and even take some exercise without any pain. The psoriasis showed some improvement, upon which the ACTH was slowly reduced to 60 mg. daily. (Total dose, 3.71 Gm. in fifty-five days. Average daily dose, 61 mg.) Several determinations of blood electrolytes showed normal values throughout.

## SUMMARY

ACTH proved strikingly effective in inducing a complete clinical and biologic remission of rheumatic symptoms and some improvement of the cutaneous lesions in 2 cases of psoriatic arthritis complicated by diabetes mellitus.

Carbohydrate tolerance was impaired, as expected. The fasting blood sugar rose to 181 mg. per 100 cc. in the first case, in which there was no

change in the PZI dosage; and to 194 mg. in the second case, in which the I-PZI dosage had to be doubled in order to keep the patient symptom-free.

The degree of improvement in arthritic symptoms was about the same in both cases, regardless of whether or not the diabetes was kept under control.

The associated diabetes mellitus did not prove to be an insurmountable contraindication to ACTH therapy for the arthritis. The results of ACTH therapy under these remarkable circumstances were so satisfactory that both patients were able to go back to work.

The psoriatic lesions, as expected, were only very slightly benefited by ACTH.

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# The Endocrine Society

## THE 1952 ANNUAL MEETING

The Thirty-fourth Annual Meeting of The Endocrine Society will be held at the Palmer House, Chicago, Illinois, Thursday, Friday, and Saturday, June 5–6–7, 1952.

The Local Chairman of Arrangements is Dr. Willard O. Thompson.

All Scientific Sessions will be held in the Palmer House. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The Annual Dinner is scheduled for Friday, June 7, at 7:30 P.M., preceded by cocktails at 6:30 P.M.

All members are urged to make hotel reservations immediately, inasmuch as the hotels expect to be filled to capacity. Make reservations directly with the Palmer House, advising time of arrival and departure date. Make your reservations now and avoid disappointment.

## 1952 POSTGRADUATE ASSEMBLY

The Fifth Annual Postgraduate Assembly in Endocrinology and Metabolism will be held at the Roney Plaza Hotel, Miami Beach, Florida, November 3–8, 1952, immediately preceding the Southern Medical Association Meeting. See the Society journals for more detailed announcement or write the Secretary.

# The American Goiter Association

## THE 1952 ANNUAL MEETING

**The next Annual Meeting of the American Goiter Association will be held in St. Louis, Missouri, May 1, 2 and 3, 1952, at the Hotel Chase. All those who plan to attend are urged to make hotel reservations immediately.**

The banquet will be held Friday night, May 2, on the Starlight Roof of the Chase, with the preceding gathering at 6 P.M. in the Zodiac Room. The business meeting will be on Friday, May 2, at 4 P.M.

Dr. Daniel L. Sexton of St. Louis, is Chairman, and Dr. Willard Barlett, Jr. of St. Louis is Co-Chairman of the Local Committee on Arrangements.

The following program ensures an interesting and instructive meeting:

### Preliminary Program

**Thursday, May 1, 1952**

8:00 A.M. *Registration* (Mezzanine—Hotel Chase)
9:00 A.M. GENERAL SESSION—Hotel Chase
W. O. THOMPSON, M.D., *President*, Presiding

1. Goiter Prevention with Iodized Salt: Results of a Thirty-Year Study.
   *Brock E. Brush, M.D.*, Detroit, Michigan
2. Some Medical-Surgical Observations on Goiter in an Endemic District.
   *Hector Perinetti, M.D., C. A. Yaciofano, M.D., L. N. Stanelone, M.D.*, and *J. Naif Nora, M.D.*, Mendoza, Argentina
3. Prolonged Administration of Iodine in the Pathogenesis of Simple Goiter and Myxedema.
   *George O. Bell, M.D.*, Boston, Massachusetts
4. The Effect of Low Iodine Diet on Thyroid Function in the Rat.
   *William L. Money, M.D.*, and *J. Edward Rall, M.D.*, New York, N. Y.
5. Differences Between Sodium 5-Iodo-2-Thiouracil and Thiouracil Plus Potassium Iodide.
   *Boris Catz, M.D.* and *Paul Starr, M.D.*, Los Angeles, California
6. Differential Diagnosis of Extrathyroidal Hypermetabolism.
   *Richard L. Rapport, M.D., Charles V. Meckstroth, M.D., George M. Curtis, M.D.* and *Sarah J. Simcox, S.B.*, Columbus, Ohio
7. *Van Meter Prize Award Paper.*

12:30 P.M.: Luncheon

2:00 P.M.: GENERAL SESSION

8. Physiology of Recurrent Laryngeal Nerve.
   *John A. Murtagh, M.D.*, and *Clarence J. Campbell, M.D.*, Hanover, New Hampshire
9. The Association of Periarthritis of the Shoulder with Hyperthyroidism.
   *Penn G. Skillern, M.D.*, Cleveland, Ohio

10. The Pathologic Effects of $I^{131}$ on the Normal Thyroid Gland of Man.
*A. S. Freedberg, M.D., G. S. Kurland, M.D.,* and *H. L. Blumgart, M.D.,* Boston, Massachusetts
11. Radioiodide in High-Risk Patients.
*William T. Salter, M.D., Katherine H. Barhydt,* and *Muriel S. Giarman,* New Haven, Connecticut
12. Investigation and Treatment of Diseases of the Thyroid Gland with Radioactive Iodine.
*Charles H. Jaimet, M.D.,* Hamilton, Canada
13. Use of Radioactive Isotopes in Management of Single Thyroid Nodules.
*Morris T. Friedell, M.D.,* Chicago, Illinois
14. Results of Treatment of Toxic Goiter with Radioactive Iodine.
*Lindon Seed, M.D.,* and *Bertha Jaffe, M.D.,* Chicago, Illinois
15. Radioactive Iodine Therapy in Carcinoma of the Thyroid
*John B. Stanbury, M.D.,* and *Gordon L. Brownell, M.D.,* Boston, Massachusetts

**Friday, May 2**

9:00 A.M.: GENERAL SESSION

16. The Influence of the Hypothalamus on the Control of Thyroid Function.
*Monte A. Greer, M.D.,* Bethesda, Maryland
17. The Metabolism of Thyroxine in Hyperthyroidism.
*Richard S. Benua, M.D.,* Rochester, Minnesota
18. The Importance of the Gastro-intestinal Tract in the Metabolism of Thyroid Hormone.
*A. Albert, M.D.,* Rochester, Minnesota
19. Rapid Changes in Plasma PBI Levels in Euthyroid and Hyperthyroid Subjects.
*B. S. Hetzel, M.D., D. S. de la Haba,* and *L. E. Hinkle, Jr., M.D.,* New York, N. Y.
20. Factors Governing the Development of the Chick Embryo Thyroid. I. Determination of the Time at Which $I^{131}$ Collection Begins.
*J. B. Trunnell, M.D.,* and *F. T. Brayer, M.D.,* Houston, Texas
21. The Effect of Vitamin C Deficiency on the Guinea Pig's Response to Thyrotrophic Hormone.
*Shirley Weisenfeld,* New York, N. Y.
22. The Physiologic Activity of Some Analogues of Thyroxine.
*J. Lerman, M.D., C. R. Harington, M.D.,* and *J. H. Means, M.D.,* Boston, Massachusetts.

12:30 P.M.: Luncheon

2:00 P.M.: GENERAL SESSION

23. *Presidential Address*
*Willard O. Thompson, M.D.,* Chicago, Illinois
24. Dosimetric Considerations in Determining Hematopoietic Damage from Radioactive Iodine.
*J. E. Rall, M.D., Charles Foster, M.D., J. Robbins, M.D.,* and *Wendell Peacock, M.D.,* New York, N. Y.
25. The Effect of X-Irradiation Upon the Iodine Accumulating Function of the Normal Human Thyroid Gland.
*M. Y. Colby, Jr., M.D.,* and *M. M. D. Williams, Ph.D.,* Rochester, Minnesota

26. Observations of the Nodular Thyroid with the Gammagraph.
*H. C. Allen, M.D., F. J. Kelley, M.D.,* and *J. A. Greene, M.D.,* Houston, Texas
27. Therapeutic Effects from Repeated Diagnostic Doses of $I^{131}$ in Adult and Juvenile Hyperthyroidism.
*Sidney C. Werner, M.D., Howard Hamilton, M.D.,* and *Martha R. Nemeth,* New York, N. Y.

**Saturday, May 3**

9:00 A.M.: GENERAL SESSION

28. The Treatment of Thyrotoxicosis from the Surgeon's Standpoint.
*Edson Fowler, M.D.,* and *Warren H. Cole, M.D.,* Chicago, Illinois
29. Postoperative Myxedema since the Advent of the Antithyroid Drugs.
*Elmer C. Bartels, M.D.,* Boston, Massachusetts
30. Recurrent Hyperthyroidism.
*Richard B. Cattell, M.D.,* Boston, Massachusetts
31. A Comparison of the Histologic Pattern of Benign and Malignant Thyroid Tumors.
*William A. Meissner, M.D.,* and *R. G. McManus, M.D.,* Boston, Massachusetts
32. The Surgeon's Dilemma: Carcinoma or Thyroiditis?
*N. J. Bellegie, M.D.,* and *Edward S. Judd, Jr., M.D.,* Rochester, Minnesota
33. Chronic Nonspecific Thyroiditis: Etiology and Pathogenesis.
*Emil Goetsch, M.D.,* and *Mildred Kamner, M.D.,* Brooklyn, N. Y.
34. Chronic Thyroiditis: A Clinical and Pathological Study of 195 Patients.
*M. E. Dailey, M.D., Stuart Lindsay, M.D., George Yee, M.D., J. Friedlander,* and *M. H. Soley, M.D.,* San Francisco, California

## THE LAURENTIAN HORMONE CONFERENCE

The Laurentian Hormone Conference of the AAAS will hold its 1952 meeting at Mont Tremblant Lodge, Mont Tremblant, Que., during the period September 7–12. The Committee on Arrangements invites applications for attendance from interested investigators and from specialists in the hormone field. Since accommodations at the hotel necessarily limit the attendance, *application must be made to the Committee on Arrangements at 222 Maple Ave., Shrewsbury, Mass.* In order to be considered, these application blanks must be received by the Committee by *June 5.*

The following program has been arranged:

I. CHEMISTRY AND BIOCHEMISTRY OF ADRENOCORTICOSTEROIDS

Synthesis of 11-oxygenated adrenal hormones from plant steroids.
*Dr. G. Rosenkrantz,* Syntex, S.A.

Hydrolysis and extraction of corticoids and 17-ketosteroids from body fluids.
*Dr. E. H. Venning,* Royal Victoria Hospital

Micro methods for the analysis of adrenocortical steroids.
*Dr. A. Zaffaroni.* Syntex, S.A.

The bioassay of adrenocorticosteroids.
*Dr. R. I. Dorfman,* The Worcester Foundation

II. ADRENOCORTICAL PHYSIOLOGY

SYMPOSIUM ON DISEASES OF ADAPTATION

The diseases of adaptation.
*Dr. H. Selye,* University of Montreal
Some experimentally produced and some naturally occurring pathologic changes in the rat.
*Drs. D. J. Ingle and B. L. Baker,* The Upjohn Company
Studies on adrenal cortical function in man.
*Dr. G. W. Thorn,* Harvard University
The adaptation concept in relation to connective tissue disease.
*Dr. W. Bauer,* The Massachusetts General Hospital
The adrenal in cancer
*Drs. C. Huggins and D. M. Bergenstal,* The University of Chicago

III. PHYSIOLOGY OF REPRODUCTION

Progesterone metabolism in the human female. Its significance in relation to reproduction.
*Dr. H. S. Guterman,* Michael Reese Hospital
The biochemistry of relaxin.
*Drs. E. H. Frieden and F. L. Hisaw,* Harvard University
Problems of fetal endocrinology. Gonadal and hypophyseal hormones.
*Dr. A. Jost,* University of Paris
The influence of steroids on uterine metabolism.
*Drs. C. Szego and S. Roberts,* University of California

IV. GROWTH HORMONE AND METABOLISM

Growth and diabetes.
*Dr. F. G. Young,* Cambridge University
The effects of anterior pituitary and adrenal cortical hormones on carbohydrate metabolism.
*Dr. R. C. deBodo,* New York University
The biosynthesis of lipids *in vitro* and its hormonal control.
*Drs. S. Gurin and R. O. Brady,* University of Pennsylvania

GREGORY PINCUS, *Chairman,*
Committee on Arrangements.

## CONGRESS OF THE INTERNATIONAL DIABETES FEDERATION

Those interested in the First International Congress of the International Diabetes Federation to be held at Leyden, Netherlands, July 7 through 12, 1952, are kindly requested to apply for a prospectus to Dr. Elliott P. Joslin, Honorary President of the American Diabetes Association, 81 Bay State Road, Boston 15, Massachusetts.

Completed application forms should be in the possession of the Executive Secretary, Dr. F. Gerritzen, 33 Prinsegracht, The Hague, Netherlands, by April 1, 1952.

## THE AMERICAN COLLEGE OF ALLERGISTS EIGHTH ANNUAL MEETING

The next annual meeting of The American College of Allergists will be held this year at the William Penn Hotel in Pittsburgh, Pennsylvania, on April 7, 8, 9. The College is offering an unusually practical program for its fellows, members and guests.

In addition to 20 addresses on general topics and special scientific investigations, there will be round tables at luncheons and sectional meetings devoted exclusively to the psychosomatic aspects of the allergic patient, allergy in infants and children, and allergic manifestations in the skin, as well as those seen in the eye, ear, nose and throat.

An innovation for meetings of allergists will be a session devoted to the problems of the allergic patient as met in modern industrial medicine. All reputable physicians are welcome to attend. For more particulars, write the College, LaSalle Medical Building, Minneapolis 2, Minnesota.

## GRADUATE INSTRUCTIONAL COURSE IN ALLERGY

On April 4, 5, 6, 1952, at Pittsburgh, Pennsylvania, The American College of Allergists will offer an instructional course in allergy. To insure complete coverage, the College has called in some 60 well known authorities in the field to give addresses, clinical talks and demonstrations. The program has been designed for physicians in other fields of practice, especially those in general practice, that they may learn to recognize and manage the allergic component in the complaints of their patients. For further information and a copy of the program, write the office of The American College of Allergists, LaSalle Medical Building, Minneapolis 2,Minnesota.